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FRACTIONATION STUDY OF BARLEY AND MALT PROTEINS[1]

BY ROBERT C. ROSE[2] AND J. ANSEL ANDERSON[3]

Abstract

The protein of O.A.C. 21 barley and of the malt produced from it were fractionated by means of various solvents and the fractions were analyzed. The results of the solubility studies and of the analyses support the use of 5% potassium sulphate for the quantitative separation of the albumin and globulin from the less soluble proteins. Fractions of the protein undissolved by potassium sulphate solution decreased in amide nitrogen and increased in arginine nitrogen with decreasing solubility. The results suggest that the protein is a complex, only part of which is soluble in 70% alcohol, and that the selection of this solution for separating it into two fractions is entirely arbitrary.

The grains of barley and malt were separated into hulls, germs and "kernels." The total weight and nitrogen content of these fractions, and the distribution of the proteins amongst them was determined. Malting causes no appreciable changes in the hulls. In the "kernel" there is a general breaking down of protein to simpler forms. In the acrospire there is an increase in non-protein nitrogen, salt-soluble protein and glutelin, but no appreciable increase in hordein. The glutelin of the acrospire differs from that of the malt "kernel" both in amide- and argenine-nitrogen content and must be regarded as a distinct protein.

I. Investigation of the Proteins of Whole Barley, Dehulled Barley and Malt

The classification of the cereal proteins is based largely on Osborne's classical work of some thirty years ago (5, 6). Since that time the gluten proteins of wheat have been studied extensively and some of the recent work (4) indicates that gluten is a complex which can be progressively fractionated, rather than a mixture of two distinct proteins as Osborne claimed. By inference, it thus seems possible that the main proteins of barley and malt may also be complexes, rather than mixtures of a prolamine (hordein) and a glutelin. Investigations designed to test this hypothesis are reported in the first part of this paper.

Materials and General Methods

The barley studied was O.A.C. 21, a small-grained, six-rowed, Manchurian variety which is used extensively for malting in Canada. Part of the sample was malted by means of the cage method under the Saladin system. The

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barley and malt contained 2.19% and 2.20% nitrogen and weighed 32.4 and 29.4 gm. per 1,000 kernels, respectively. All samples were ground in a Wiley mill fitted with an 0.5 mm. sieve.

Protein fractions were hydrolyzed in 20% hydrochloric acid for 24 hr. Non-protein compounds were hydrolyzed in 2 *N* sulphuric acid as recommended by Vickery and Pucher (8).

Total nitrogen was determined by the Kjeldahl method. Amide nitrogen was determined by aerating for 2 hr. at 45° C. after titrating to phenolphthalein with sodium hydroxide and saturating with magnesium oxide, and the residue from this determination was used for amide-nitrogen analyses according to the method of Plimmer and Rosedale (7).

Studies with Salt and Alcohol Solutions

Salt-soluble proteins of all cereals are commonly determined by extracting with 5% potassium sulphate, but Blish (2) has pointed out that the selection of this solution is quite arbitrary. The results of some comparisons of the action on barley proteins of this solution and those of other salts commonly in use therefore appear to be worth reporting.

Separate portions of whole barley meal were subjected to five successive extractions of 15 min. each, at room temperature, with chloroform water followed by 5% potassium sulphate, chloroform water followed by 5% magnesium chloride, and chloroform water followed by 8% potassium iodide, and in each case the residue was extracted three times, for 30 min. each, with 70% alcohol in a sealed tube at 82° C. The percentage of the total nitrogen extracted by these methods was determined and the results are presented in Table I.

TABLE I

SOLUBILITY OF PROTEINS OF WHOLE BARLEY MEAL

Initial solvent	Nitrogen extracted by initial solvent, % of T.N.	Nitrogen subsequently extracted by hot 70% alcohol, % of T.N.	Total nitrogen extracted, % of T.N.
Water	19.0	36.2	55.2
5% K_2SO_4	31.6	36.4	68.0
5% $MgCl_2$	36.7	31.2	67.9
8% KI	41.8	27.0	68.8

The data show that although 5% potassium sulphate extracted 12.6% more nitrogen than did water, hot alcohol extracted an additional 36% in each case. The protein extracted by potassium sulphate therefore appears to be insoluble in alcohol. The magnesium chloride and potassium iodide solutions extracted more nitrogen than potassium sulphate but the total nitrogen extracted by the salt and alcohol solutions was not substantially increased. It appears therefore, that 5% potassium sulphate extracts a distinct fraction, whereas the other salt solutions cut across two fractions properly differentiated as salt-soluble and alcohol-soluble.

A number of fractions of barley and malt proteins were prepared and analyzed for amide and arginine nitrogen. Duplicate amounts of whole

meal were extracted successively with water, 5% potassium sulphate and hot 70% alcohol. A second pair of samples was extracted successively with water, 5% potassium sulphate, 30%, 50% and hot 70% alcohol. Owing presumably to more serious denaturation, these successive extractions with increasing concentrations of alcohol did not extract as much protein as one treatment with hot alcohol, and in order to obtain the fraction which was least soluble in alcohol, two more samples were extracted successively with 5% potassium sulphate, 60% alcohol and 70% hot alcohol.

Water extracts were treated with sufficient trichloracetic acid to bring them to 2.5% in respect to this reagent, and after standing over night the protein precipitate was separated by centrifuging. Salt-soluble protein was precipitated by adding sufficient sodium tungstate to give a 1% solution and acidifying with sulphuric acid after the manner of Hiller and van Slyke (3). The alcohol extracts were acidified with hydrochloric acid and boiled to remove the alcohol and concentrate the solution. Nitrogen determinations were made by the methods previously described.

The analytical results are reported in Table II. They show that distinct differences in amide- and argenine-nitrogen content exist between albumin, globulin, hordein and glutelin. When hordein is progressively fractionated, however, it is shown that, with the exception of the most soluble protein

TABLE II

AMIDE AND ARGININE NITROGEN CONTENT OF WHOLE BARLEY AND MALT PROTEIN FRACTIONS

Fraction	Barley			Malt		
	Nitrogen range of fraction, % of T.N. of meal	Amide N, % of T.N. of fraction	Arginine N, % of T.N. of fraction	Nitrogen range of fraction, % of T.N. of meal	Amide N, % of T.N. of fraction	Arginine N, % of T.N. of fraction
Non-protein	0.0 – 9.0	11.2	15.0	0.0 – 21.4	10.6	16.2
Water-soluble protein	9.0 – 19.0	9.2	18.5	21.4 – 37.2	8.7	17.2
5% K_2SO_4-soluble protein	19.0 – 31.6	9.1	22.0	37.2 – 50.0	8.5	21.6
Hot 70% alcohol-soluble protein	31.6 – 68.0	21.0	9.4	50.0 – 74.8	20.6	9.1
Residue	68.0–100.0	10.0	13.6	74.8–100.0	8.0	13.8
Alcohol-soluble fractions						
30% alcohol after H_2O and K_2SO_4	31.6 – 36.8	18.7	11.0	50.0 – 53.2	18.4	14.0
50% alcohol after H_2O, K_2SO_4 and 30% alcohol	36.8 – 50.8	23.5	6.8	53.2 – 61.4	23.5	8.2
Hot 70% alcohol after H_2O, K_2SO_4, 30% and 50% alcohol	50.8 – 65.0	20.2	9.0	61.4 – 69.2	19.6	10.2
Hot 70% alcohol after H_2O, K_2SO_4 and 60% alcohol	59.3 – 67.8	17.8	11.5	68.2 – 72.4	15.6	12.3

which is probably contaminated with small amounts of salt-soluble protein, the fractions decrease in amide nitrogen content and increase in argenine nitrogen content with decreasing solubility, thus approaching the composition of glutelin. If glutelin could be fractionated for analysis it seems possible that it would be found that the fractions increase in amide nitrogen and decrease in argenine nitrogen with increasing solubility and thus approach the composition of the least soluble fraction of hordein. Attempts to demonstrate this experimentally, reported below, were unsuccessful since relatively unchanged fractions of glutelin could not be prepared. Nevertheless, although the evidence is by no means complete, there are some grounds for believing that the main protein of barley or malt is a complex and that the division of it into two fractions by means of hot 70% alcohol is purely arbitrary.

Studies with Acid and Alkaline Solutions

A study was made of the effect on glutelin of aqueous and alcoholic solutions of hydrochloric acid and sodium hydroxide, by methods of extraction similar to those reported above. Alcoholic solutions were more efficient and were more easily employed because they did not swell the starch. At room temperature 0.05 *N* sodium hydroxide in 70% alcohol dispersed about one third of the glutelin and 0.1 *N* and 0.2 *N* solutions dispersed little more. By raising the temperature to 82° C., almost complete dispersion was obtained. Hydrochloric acid was not as effective. At 82° C. the extraction curve was almost identical with that obtained with sodium hydroxide at room temperature. A 0.1 *N* solution of acetic acid dispersed very little of the glutelin fraction even at 82° C. It was readily demonstrated, as could have been predicted, that with these concentrations of reagents and particularly at the higher temperature, amide nitrogen was hydrolyzed from the protein, and suitable fractions of glutelin for analysis therefore could not be prepared by means of these reagents. It is worth noting, however, that just as the hordein-glutelin complex can be divided at any point in the hordein range by choosing the right concentration of alcohol and the right temperature, so can it be divided at any point in the glutelin range by choosing a suitable temperature and concentration of sodium hydroxide in 70% alcohol.

Studies with Sodium Salicylate Solutions

Attempts were made to fractionate the proteins of barley and malt by means of sodium salicylate solutions. It appeared that the use of this solution, which has been used effectively in studying wheat proteins, might serve not only to produce suitable fractions of the least soluble proteins for analysis but also to provide further evidence respecting the solubility of the other proteins. Barley proved to be far less amenable than wheat: comparatively pure protein, corresponding to washed wheat gluten, cannot be obtained, and studies of the whole meal were made difficult by the low concentration of the protein and the interfering action of the starch, which swells in concentrated solutions of sodium salicylate.

Since it was not possible to produce by means of this reagent suitable progressive fractions of hordein and glutelin for analysis, the investigations as a whole proved fruitless. Nevertheless, a selection of the solubility studies appears to merit brief description.

Samples of whole barley meal of such size as to contain 50.0 mg. of nitrogen were extracted at room temperature on a mechanical shaker, first with 50 ml. of solution for 3.5 hr. and then with three 25 ml. portions for 0.5 hr. each. The extractions were carried out with various concentrations of sodium salicylate solution, and the percentage of the total nitrogen extracted by each concentration was determined. The process was also repeated with whole-malt meal and meal prepared from barley from which the hulls and germs had been removed by means of a wire-brush mill. The results are shown in Fig. 1 in the form of a graph.

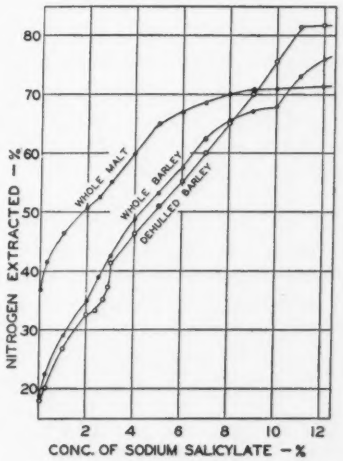

FIG. 1. *Extraction of barley and malt with sodium salicylate solutions.*

The definite irregularity in the curve for dehulled barley, and the slight irregularity in that for malt, beginning at about 2% sodium salicylate indicate differences in the solubility of the protein extracted before and after this point. Since this concentration of sodium salicylate extracts approximately the same percentage of nitrogen from the respective products as 5% potassium sulphate, these results support the earlier conclusion that the latter reagent serves to divide the proteins at a logical point.

No particular importance is attached to the irregularity in the curve for whole barley at 10% sodium salicylate. It was noted that this solution swelled the starch but did not disperse it, whereas a 12% solution dispersed it to such an extent that it had to be decanted with the extract and in all probability it carried some undispersed protein with it and thus produced the irregularity in the curve. In experiments with dehulled barley the amount of starch decanted with the extracts appeared to be proportional to the concentration of sodium salicylate, and thus no irregularity was created.

II. The Distribution of Proteins Amongst the Physical Fractions of Barley and Malt

Current methods of fractionating barley and malt proteins, in which the whole of the kernel is ground up before extracting with various solvents, take no cognizance of differences in the distribution of the protein in various parts of the kernel or of possible qualitative differences in proteins of the

same solubility occurring in different parts of the kernel. The literature on malting appears to contain no useful body of data on this subject, and because of its importance in elucidating the changes which take place during malting, and of its bearing on the validity of the methods now used in studying barley and malt proteins, preliminary investigation of it was undertaken.

Materials and Methods

The materials and the methods used for extraction, hydrolysis and nitrogen determinations were identical with those described previously.

The separation of the physical fractions of the whole grains was carried out as follows:—The hulls and embryos were separated from the "kernels" by means of a wire-brush mill. The "kernels," hulls and germs of barley were then separated by sifting and fanning. It was necessary to resort to hand picking in order to separate the malt acrospires from small pieces of "kernel."

Total Weight and Nitrogen Content

The results of the determinations of the total weight and nitrogen content of the various physical fractions of barley and malt are reported in Table III. The hulls of both barley and malt, though forming a considerable portion of

TABLE III

TOTAL WEIGHT AND NITROGEN CONTENT OF PHYSICAL FRACTIONS OF BARLEY AND MALT
(All figures reported on dry basis)

Physical fraction	Weight		Nitrogen		
	gm. per 1000 kernels	% of whole grain	% of fraction	mg. per 1000 kernels	% of T.N. of whole grain
Whole barley	32.4	100	2.19	710	100
Hulls	3.9	12	0.40	16	2.2
Germs	0.63	2	6.30	40	5.6
Kernels*	27.9	86	2.34	654	92.2
Whole malt	29.8	100	2.20	656	100
Hulls	3.9	13	0.41	16	2.4
Acrospires	1.4	5	5.32	75	11.4
Kernels*	24.5	82	2.31	565	86.2

**Whole grain less hulls and germs. Values obtained by difference.*

the whole grain, are low in nitrogen and thus contain only a small proportion of the total nitrogen. The germs, on the other hand, are high in nitrogen and thus, particularly in malt, contain a very appreciable proportion of the total nitrogen. For these reasons alone, studies made with the whole grain must fail to present a clear picture of the changes which take place during malting in the most important part of the kernel, that is, the endosperm.

Distribution of the Proteins

The distribution of the proteins amongst the physical fractions of barley and malt, as shown by determinations of the total nitrogen of protein fractions extracted by the classical solvents, is shown in Table IV.

TABLE IV

DISTRIBUTION OF BARLEY AND MALT PROTEINS

Nitrogen fraction	Barley				Malt			
	Kernels*	Hulls	Germs	Whole barley	Kernels*	Hulls	Germs	Whole malt
Nitrogen in mg. per 1000 kernels								
Non-protein	53	5	6	64	117	7	16	140
Water-soluble	58	2	11	71	91	1	11	103
5% K_2SO_4-soluble	87	0	3	90	81	0	3	84
Hot 70% alcohol-soluble	253	1	3	257	158	1	4	163
Residue	203	8	17	228	118	7	41	166
Total	654	16	40	710	565	16	75	656
Nitrogen as % of total nitrogen of fraction								
Non-protein	8	31	16	9	21	43	22	21
Water-soluble	9	13	28	10	16	7	15	16
5% K_2SO_4-soluble	13	0	7	13	14	2	4	13
Hot 70% alcohol-soluble	39	6	6	36	28	9	5	25
Residue	31	50	43	32	21	39	54	25

**Whole grains less hulls and germs. Values obtained by difference.*

The data show that malting affects different parts of the grain in different ways. Practically no change occurs in the hulls. In the "kernel" there is a general breaking down of protein into simpler and more soluble forms. This results in large increases in the non-protein and water-soluble protein which take place at the expense of the hordein-glutelin fraction. The amount of salt-soluble protein remains almost unchanged. On the other hand, this process is reversed in the germ. It is true that the non-protein nitrogen is increased, but the protein itself is also built up into the least soluble form, the amounts of water-soluble, salt-soluble, and alcohol-soluble protein remaining almost unchanged. The results of the investigation demonstrate that a more accurate picture of the changes which occur during malting can be obtained by separating the grain into its various physical fractions before analysis.

The results of the analyses of whole barley and whole malt agree with those of Bishop (1) except that he found that the salt-soluble protein increased during malting. This difference can probably be accounted for by differences in the barleys and the malting methods used in the two investigations. Bishop found that, during malting, glutelin first decreased and then increased. The increase was attributed to re-synthesis in the embryo and rootlets and the

first part of this hypothesis is supported by the results of the present investigation. It was also suggested that the decrease in the rate of disappearance of hordein might be due to re-synthesis in the embryo. This suggestion is not substantiated by the data presented in Table IV, which show that the acrospires contain little alcohol-soluble protein.

Analyses of Acrospire Proteins

As several grams of acrospire meal were available and as this fraction is high in nitrogen, it was possible to obtain some protein fractions of it for arginine and amide nitrogen analyses. There was so little of the salt-soluble and alcohol-soluble fractions that it was impossible to analyze them, but the results for the other fractions are reported in Table V.

TABLE V

AMIDE AND ARGININE CONTENT OF MALT ACROSPIRE NITROGEN FRACTIONS

Fraction	Amide N, % of T.N. of fraction	Arginine N, % of T.N. of fraction
Non-protein	12.6	22.4
Water-soluble	7.6	21.2
Residue	5.5	24.4

The data are extremely interesting. If they are compared with similar data for the proteins of whole barley and malt given in Table II, it will be observed that the acrospire proteins are entirely different. The residue protein is, in fact, so different in both its argenine- and amide-nitrogen content that it must be regarded as a distinct protein rather than as malt glutelin. In these circumstances it is not surprising that some difference of opinion has existed in the past on whether the proteins extracted from whole barley and malt were identical. It appears that by suitable fractionation it can be demonstrated that they are not identical.

Acknowledgment

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RHIZOPHIDIUM GRAMINIS N. SP., A PARASITE OF WHEAT ROOTS[1]

By G. A. Ledingham[2]

Abstract

A Chytrideaceous parasite apparently not hitherto described has been found on the roots of wheat seedlings grown in soil from Ottawa, Ontario; and Boston, Massachusetts. The fungus is closely related to the uniporous Rhizophidiums but since it does not appear to be identical with any of these morphologically and exhibits a type of sexuality not previously described in this genus it is considered to be a new species. The name *Rhizophidium graminis* is proposed.

Introduction

In the past, mycologists have reported a number of species of *Rhizophidium* growing on algae, aquatic fungi, lower animals and pollen grains in water. As far as the writer is aware, no species of this genus has been described as growing on the living roots of higher plants. Hence the present species, parasitic on such roots, is of additional interest; it is moreover apparently a new species, and for these reasons the following notes concerning it are presented in the hope they may prove of interest to other workers in the same field.

Materials and Methods

In 1930, a few sporangia of this *Rhizophidium* growing as a parasite on the roots of wheat were noticed while examination was being made for an intracellular root parasite in the same host. Infection was in general light and very scattered, but at times, and on certain specimens, the fungus was plentiful.

Preliminary studies were made at the University of Toronto in the spring of 1932, with material which was found infecting roots of wheat grown in soil from the Central Experimental Farm, Ottawa. During the fall of the same year further observations were made at the laboratories of Cryptogamic Botany of Harvard University, using material brought from Ottawa, and additional material was provided from naturally infected plants of a species of *Panicum* growing by a small pond in the grounds of the Arnold Arboretum, Boston. When Marquis wheat was grown on soil in which this grass was present, excellent infection was obtained in two to three weeks.

Most of the studies were made on living material in water mounts, but in some cases lacto-phenol was used as a preservative. Mounts stained with cotton blue or acid fuchsin in this medium were particularly useful in demonstrating the extent of the rhizoidal system.

[1] *Manuscript received, February 5, 1936.*

This study was begun in the Botany Department, University of Toronto, during the tenure of a National Research Council Scholarship and a research assistantship in the Department; it was continued in the Laboratories of Cryptogamic Botany, Harvard University, and completed in the National Research Laboratories, Ottawa. Listed as Contribution No. 128 of the Laboratories of Cryptogamic Botany, Harvard University.

[2] *Biologist, National Research Laboratories, Ottawa, Canada; formerly Research Assistant, Harvard University.*

Diagnosis

As the fungus from these two sources, when investigated intensively, proved to be the same species and showed definite points of distinction in morphology as well as in host habitat, differentiating it from the species already reported, there seems to be justification for describing it as new with the following diagnosis:

Rhizophidium graminis sp. nov.

Zoosporangiis extramatricalibus solitariis vel gregariis maturitate 10–75 μ diametro globosis vel ellipsoideis, levibus, basi filamentis myceleis tenuibus praeditis, eisdem in cellula matricis penetrantibus; zoosporis numerosis 3 × 1.8 μ, apice zoosporangeorum fracto erumpentibus, guttula hyalina praeditis atque basi attenuato cilio uniquo; sporangii perdurantibus 6–12 μ diam. globosis, endosporis levibus et exosporis asperis.

Rhizophidium graminis n. sp.

Zoosporangia 10–75 μ in diameter, globose to ellipsoid, with smooth walls, solitary or gregarious, giving rise at the base to fine rhizoids which ramify within the host cell. Zoospores 3 × 1.8 μ uniguttulate and uniciliate, the cilium 10–12 μ in length, liberated in large numbers by the rupturing of the apex of the sporangium. Resting spores 6–12 μ diameter, globose with smooth endospore and rough scabrous exospore wall and oily protoplasmic contents, sexual fusions occurring between rhizoids of two thalli. Germination of the spore takes place by the extrusion of the cell contents to form a thin-walled sporangium.

Parasitic on roots of *Triticum* and *Panicum* spp. in Ontario and Massachusetts.

Specimens have been deposited in the Farlow Herbarium, Harvard University and the Mycological Herbarium, Department of Botany, University of Toronto.

Observations on the Morphology of the Fungus

The Zoosporangial Phase

This phase is initiated when the amoeboid zoospore attaches itself to the root hair or to the epidermal cell of the wheat root and begins to develop directly into the zoosporangium. The youngest stage found, about 4 μ in diameter, contains a fine-grained protoplasm in which are embedded numerous larger particles and several vacuoles (Fig. 1).

The rhizoidal system, which supplies the growing thallus with food from the host, develops as a small outgrowth which arises from the side of the zoospore in contact with the host cell. After the primary rhizoid has penetrated the host-cell wall, growth continues, the rhizoids at first being rather short, thick and unbranched, with blunt terminals. But later, owing to their profuse branching they develop into a densely woven mass of very fine threads (Fig. 2). There is no evidence of a subsporangial vesicle or any enlargement at the base of the zoosporangium such as one finds in the genus *Phlyctochytrium*.

Meanwhile growth of the sporangium has been taking place simultaneously with the development of the rhizoids. The vacuoles and coarse granules characteristic of early stages have disappeared and the content has become homogeneous (Fig. 2). Numerous refringent granules next appear, making it very difficult to follow the process of zoospore delimitation. Ultimately the mature sporangium is closely packed with these oil-like droplets, one of which is included in each zoospore. During the process of zoospore formation there is very little movement of the contents of the sporangium, and even after the zoospores are fully formed they remain motionless, with no suggestion as to when discharge is about to take place. The sporangia show great variations in size at maturity, measuring as little as 10 μ and as much as 100 μ in diameter and containing, according to their size, from 8 to more than 100 zoospores.

Zoospore discharge was controlled most successfully by varying the amount of soil moisture. If the soil was allowed to become slightly dry, zoosporangia developed and matured normally but active zoospore discharge did not take place. In material kept under these conditions for several hours there was an accumulation of ripe sporangia which, when placed in water, released zoospores almost immediately.

Zoospore discharge is of a characteristic type in this species in that the wall of the sporangium bursts suddenly, forcibly ejecting the zoospores. These remain in a quivering mass for a moment, then swim away in all directions. The empty cup-shaped basal wall of the sporangium with its irregularly torn upper margin persists on the host-cell wall for several days (Fig. 6). Occasionally the tear may proceed regularly, leaving a cap-like structure (Fig. 5). The persistent nature of this wall is in contrast to the evanescent character of the same in *R. clinopus*, which according to Scherffel (4) lasts only a few hours.

It is quite possible to distinguish *Rhizophidium graminis* from other species of the genus by this characteristic method of zoospore discharge. In other species discharge takes place through pores, as in *R. pollinis*, or by exit tubes as in *R. transversum*, or by gelatinization of the tip of the sporangium as in *R. clinopus*. There is no indication in *R. graminis* that the zoospores are surrounded by a vesicle or embedded in "slime" as are those of *R. sphaerocarpum* described by Sparrow (7, p. 519), when they are freed from the sporangium.

Active zoospores are usually pyriform in shape and average 3 by 1.8 μ in size. At the blunt anterior end lies the conspicuous refringent granule referred to previously. The fact that this drop blackens in osmic acid, dissolves in ether and reacts to fat stains, indicates that it is of an oily nature.

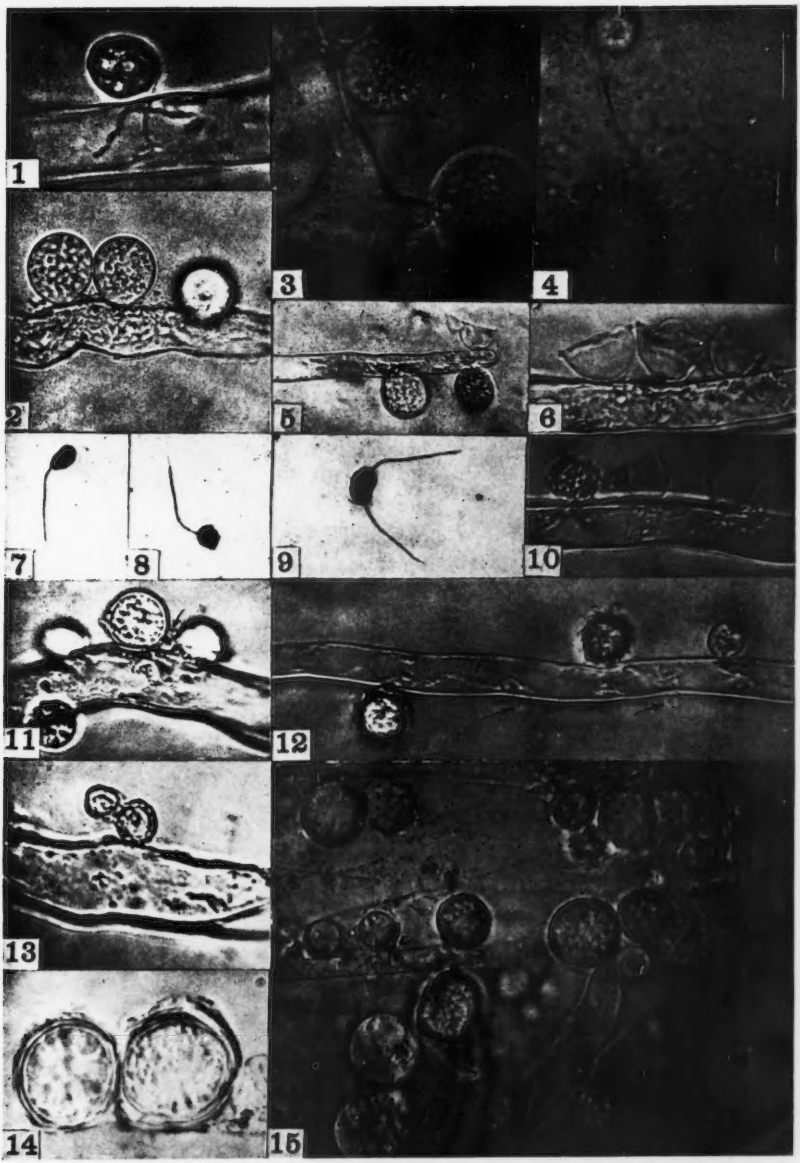
Zoospores may be readily stained by using Cotner's method (1). They usually become nearly spherical when killed in osmic acid fumes (Fig. 8), but some retain their original form (Fig. 7). Near the anterior end of the spore occur several coarse granules which stain heavily. The nucleus lies near the posterior end, and close by is the blepharoplast from which arises the long, straight conspicuous cilium, 10 to 12 μ in length.

On many occasions, in stained preparations and also in hanging drop mounts, biciliate swarmers twice the usual size were found. They were conspicuous because of their awkward rolling motion when swimming. As biciliate zoospores were never seen at the time of zoospore emission, or for some time after, it would seem that they must arise as a result of fusion between two zoospores, rather than from incomplete cleavage. Observations on the process of fusion in hanging drop mounts supports this view. Fusion takes place in the same manner as described by Kusano for zoospores of *Synchytrium fulgens* (3). Prior to fusion the zoospores creep about sluggishly and send out pseudopodia. Sometimes two touch but break apart again. When first joined, only a small part of the surface of one is in contact with the other. The two cilia are at opposite poles, two refringent granules are present, and the line of fusion is marked by a deep constriction. This constriction gradually disappears, the oil globules fuse and the spore becomes ovoid in shape, measuring 4.5 by 2.5 μ (Fig. 9).

The Sexual Fusion

Zoosporangia of *R. graminis* are produced by the enlargement of a single zoospore which has developed rhizoids within the host cell. No sexual mechanism is concerned in the production of this phase, nor indeed is it to be expected. In the resting spore which appears somewhat later it seemed logical, judging from the course of events in many related organisms, to expect a fusion somewhere during development. When an occasional fusion between two zoospores was first found it was thought that perhaps the resulting biciliate spore was a zygote, which after infection would give rise to a resting spore. However, when a small, undeveloped thallus, almost devoid of contents, was found attached to the host near an immature resting spore, doubt arose as to whether this was the correct interpretation to put on such fusions. By careful searching near many resting spores it was found that this empty cell was generally present. Sometimes it lay against the resting spore close to the base (Fig. 11), but it always remained an independent body, not connected in any way as is the companion cell found in *Rhizophidium globosum* (2). The contents, therefore, could not have passed from one to the other except through the rhizoids, which were closely intermingled. It was found that even where the two thalli were some distance apart the rhizoids were in contact with one another (Fig. 12). Undoubtedly transfer of nuclear material from one to the other had been accomplished through the rhizoids. The empty cell might, therefore, be considered a male gamete, which had fertilized the female now developing into a resting spore.

Petersen (4) described a type of fusion similar to this in *Siphonaria variabilis* in 1903. Dr. F. K. Sparrow who has recently observed the same phenomenon in *Rhizoclosmatium* and verified Petersen's observations on *Siphonaria*, kindly examined some material of *Rhizophidium graminis* and called the attention of the writer to the similarity.



Rhizophidium graminis. All figures except 7, 8 and 9 from living material. FIG. 1. *Young zoosporangium.* ×*1300.* FIG. 2. *Zoosporangia prior to segmentation into zoospores.* ×*455.* FIG. 3. *Mature zoosporangia.* ×*790.* FIG. 4. *Same as Fig. 3, a few minutes later.* ×*790.* FIG. 5. *Empty zoosporangium and two others beginning to form zoospores.* ×*455.* FIG. 6. *Remnants of zoosporangia after zoospore discharge.* ×*1300.* FIG. 7. *Stained zoospore.* ×*1300.* FIG. 8. *Stained zoospore.* ×*1300.* FIG. 9. *Stained biciliate zoospore formed as a result of fusion of two uniciliate spores.* ×*1300.* FIG. 10. *Resting spore and remnants of two zoosporangia.* ×*1300.* FIG. 11. *Resting spore with male cell, indicated by arrow, lying close to it.* ×*850.* FIG. 12. *Resting spores, with male cells, indicated by arrows, located at a distance.* ×*1300.* FIG. 13. *Germinating resting spore.* ×*1300.* FIG. 14. *Two resting spores, part of a row crowded together on an epidermal cell.* ×*1366.* FIG. 15. *Resting spores on an old dried wheat root.* ×*790*

The Resting Spore

The mature resting spores vary from 6 to 12 μ in diameter, and there is some correlation in size and, indeed, in shape, with the location on the host, for the smaller spores are generally developed on root hairs and occur singly and scattered, so that they attain a spherical shape, whereas the larger ones develop on the cells of the epidermis, and usually occur in groups or in rows, so closely packed that their sides are flattened.

In the dense protoplasm of these resting spores numerous refringent granules are present (Figs. 12, 14 and 15) the coarseness of which distinguishes them from the finer granules characteristic of the zoosporangium (Figs. 2, 3 and 5), but there is no large central oil globule such as is found in the resting spores of *Rhizophidium pollinis* (2).

Two walls are present, a smooth hyaline inner one about 0.5 μ in thickness, formed at an early stage in the development of the spore and an outer one, developed later, generally irregularly rough and scabrous (Figs. 12 and 13), but in some old spores in disintegrating root material, appearing almost smooth (Fig. 15).

The complete process of germination has not been observed. However, when spores which had been frozen in ice for several weeks were returned to conditions favorable for growth, the content in one instance was extruded into a thin zoosporangium-like sac, which increased in size for several hours, until it had attained dimensions almost equalling those of the resting spore itself, as shown in Fig. 13. Even though the formation of zoospores in this extruded material did not take place it seems logical to assume that this represented the preliminary stages of germination, for the process was very similar to that described by Sparrow for *Chytridium schenkii* (6).

Conclusion

Several attempts have been made to cultivate *R. graminis* on various culture media but without success. Until some method is obtained of growing the fungus in pure culture it will be difficult to test its pathogenic effects on the host. The present observations, though perhaps not too adequately worked out, are presented in the hope that they will be of interest to others studying the root parasites of plants.

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The writer wishes to thank Professor D. L. Bailey and Professor H. S. Jackson of the Botany Department, University of Toronto, Professor W. H. Weston, Jr. and Dr. D. L. Linder of the Laboratories of Cryptogamic Botany, Harvard University, and Dr. F. K. Sparrow of Dartmouth College, for many helpful suggestions.

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STUDIES ON DROUGHT RESISTANCE IN SPRING WHEAT[1]

By O. S. Aamodt[2] and W. H. Johnston[3]

Abstract

The results of a number of preliminary investigations regarding the nature of drought resistance in wheat are presented.

A study of the susceptibility of wheat plants to drought at different stages of development showed that the shooting and the soft-dough periods were the most critical, from the point of view of grain yield. Heavy foliage losses occurred when plants were exposed during the shooting period, and recovery was very slow. Exposure of plants during the stooling stage resulted generally in transient injury, as the capacity for recovery was particularly great at this time. Plants subjected to drought during the hard-dough stage sacrificed little in kernel plumpness.

Hardening of wheat plants by soil drought, or by limited exposures to atmospheric drought, increased their resistance to exposures of severe atmospheric drought.

The drought-resistant varieties Milturum and Caesium were found to possess a more highly branched primary root system than the non-resistant varieties Marquis and Reward. The durum varieties, Pentad and Pelissier, were found to excel in numbers of primary roots produced, having an average of five per plant. Milturum, Caesium and Baart had more than four primary roots per plant on the average; while Canus, Federation, Reward, Marquis, Garnet and Red Bobs No. 222 had from 3.2 to 3.6. Reliance wheat and wild oats had not more than three primary roots per plant.

The addition of superphosphate to the soil resulted in a slight decrease in number of primary roots of Milturum, Caesium and Marquis, and a slight increase in the number of Reward.

The kernels of the drought-resistant varieties, Milturum and Caesium, did not show any marked superiority to those of Marquis, Reward, Garnet and Red Bobs No. 222, in capacity to germinate in the more concentrated solutions of sodium chloride, potassium chloride and sucrose. A higher energy of germination was displayed by the kernels of Milturum, Caesium and Garnet than by those of the other three varieties tested.

Milturum and Canus exhibited high resistance to injury from windburn when tested in the late stooling to early shooting growth stages. The durum varieties Pentad and Kubanka and the soft wheat, Baart, on the other hand, showed high susceptibility. The soft wheat, Federation, and the variety Red Bobs No. 222 were moderately susceptible.

Introduction

Drought is probably the major limiting factor in crop production in the semi-arid regions of the world. Within these regions are found the principal wheat-producing areas of Canada, United States, Argentine, Australia and Russia. Periodically these semi-arid areas are subjected to conditions of drought, which may be so severe as to cause a virtual crop failure. The disastrous harvest of 1921 in Russia due directly to drought conditions caused a national calamity, and destroyed by famine and accompanying disease millions of human lives (35). The same country suffered a drought of even greater intensity in 1892 (35). There is seldom a year in which some part of

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the wheat-growing areas of the world is not subjected to drought of varying intensity. It has been estimated that the total yield of wheat on the Canadian prairies during the five years, 1930–34, has been reduced on the average by approximately 75,000,000 bushels. This loss is equal to that resulting from black stem rust in epidemic years.

Breeding for drought resistance in cereals has received little attention from investigators, except in Russia, where it received great impetus after the drought of 1921 (35). In Russia, the nature of drought resistance and its application to breeding problems are being studied. Already several new drought-resistant wheat varieties, superior in yield and quality to the older Russian varieties, have been developed by the plant breeders of that country (41, 43).

It is only comparatively recently that research projects have been developed in America to combat drought. In 1928 the University of Alberta received a number of Russian wheat varieties that had been developed for areas of low rainfall. A number of these proved to be superior to local varieties in resistance to drought, but were inferior in certain agronomic characters, as well as in quality. In 1929 a breeding investigation of drought resistance was started when crosses were made between the drought-resistant Russian varieties and the improved local varieties. In 1931, financial assistance was received from the National Research Council of Canada, and a comprehensive programme for the study of drought resistance was begun. The following fourfold programme is being followed:

(a) To ascertain the resistance of wheat varieties to drought.

(b) To study in detail certain morphologic and physiologic characters associated with drought resistance.

(c) To study the mode of inheritance and genetics of drought resistance.

(d) To produce desirable strains of cereals resistant to drought.

The results discussed in this paper pertain to preliminary investigations conducted regarding sections *(a)* and *(b)* of the programme outlined above.

The results of certain investigations conducted on the *(c)* and *(d)* phases of the problem have been summarized by Aamodt and Torrie (4).

Literature Review

Although considerable work has been conducted on the physiology of drought resistance in plants, little has been accomplished in the way of breeding resistant varieties. A possible explanation for this state of affairs is the failure of investigators to recognize the complex nature of drought resistance. The bulk of the work reported represents attempts to find some simple index of measuring drought resistance by means of a number of relatively easily observable anatomical or physiological characteristics. The more modern school of thought, as represented by Maximov (26), believes that the only true criterion of drought resistance is the ability to endure drought with-

out injury. Drought resistance possessed by any group of plants is due to a multiplicity of morphological and physiological characteristics, which investigators have, so far, failed to separate into their component parts.

The difficulty of obtaining a true conception of what is meant by drought resistance is complicated at the outset by the existence of two types of drought, each of which presents to the investigator a distinct series of problems. The type of drought that is best known, and which has received the greater amount of attention in the past, is that occurring when, under normal atmospheric conditions or otherwise, the soil ceases to provide the plant with sufficient moisture to take the place of that lost by transpiration. Drought of this nature is commonly known as soil or edaphic drought. The second type of drought, which appears to have gained recognition only relatively recently, is known as atmospheric drought, and is usually caused by hot dry winds, which may produce desiccation of the plant, even under conditions of relatively high soil moisture. Shantz (39) in his work on water requirements of plants gave special attention to the soil type of drought. Vasiliev (48) has stated that there appears to be no ground for believing that it is possible to breed plants resistant to soil drought, while the breeding of plants resistant to atmospheric drought should be possible.

Drought resistance has been found to be a variable factor and depends to a large extent upon environmental conditions prevailing before drought sets in. Tumanov (44) found that repeated wilting of sunflowers resulted in a "hardening" process analogous to that observed at low temperatures (17, 30, 31). Kondo (23) observed the same phenomenon to occur in the case of soybeans as well as in sunflowers. Kondo also found that plants grown under conditions of insufficient soil moisture, suffered considerably less injury from exposure to severe conditions of soil drought than plants grown under conditions of optimum soil moisture. Tumanov (45) noted that oat plants grown in the shade were less resistant to soil drought than plants grown in direct sunlight.

Even the different organs of the same plant have been found to vary in their degree of drought resistance. Bartholomew (7) found that the suction tension developed in the leaves of wilting lemon plants withdrew water from the fruit, causing a pathological disturbance known as "internal decline of lemons". Lloyd (25) noted that a similar phenomenon caused the dropping of young buds of cotton plants. Tumanov (45) found that leaves of sorghum withdrew water from the stem under stress of soil drought, while the leaves of buckwheat lacked this ability. This investigator also found that the aerial parts of the alfalfa plant exhibited less drought resistance than did the crown and the roots. Krasnosselsky-Maximov (24) cites instances in which the leaves of cereals drew water from the inflorescences when under exposure to artificial dry winds. She also observed that when the leaves were removed before exposure of the plants to this wind, the inflorescence had a much higher water content at the end of the exposure than inflorescences of plants with the leaves present.

It is also well known that plants exhibit different capacities for enduring drought conditions at various stages of their development. The mature seed can undergo almost complete desiccation, without apparent harm. This resistance appears to be manifested also for the first few days following germination. Beal, cited by Robbins (38), germinated seeds of wheat and buckwheat six times, each time allowing the root and stems to grow to the length of the grain before stopping the process by dryness. He found that five repeated germinations were necessary before the percentage germination of these seeds was materially reduced. With the development of leaves, however, the plants became susceptible to severe injury by desiccation. Brounov (26) found that cereals were most susceptible to drought during the period of rapid growth of the culm prior to heading. He applied the term "critical period" to the stage in which the plant was most susceptible. Moliboga (29), Pullman (26), Azzi (6) and Beauverie (8) have also studied the critical periods in cereals and have confirmed the findings of Brounov.

To the plant breeder the study of critical periods in their relation to peculiarities of local climate is of utmost importance. Only those varieties should be selected for a given area, whose critical periods do not coincide with the greatest combination of adverse meteorological factors. Ivanov (20) cites the example of Turkestan wheat, which develops very rapidly during the early stages of growth and comes into head long before other types. The second stage is slow, thus enabling the plant to withstand any early drought, and utilize late rains. Talanov (43) found that the variety Milturum 0.321, owing to its long stooling period (a relatively drought-resistant stage), was able to withstand early summer drought and avail itself of late rains and more suitable atmospheric conditions.

These examples serve to show that it is possible for plants, by means of a delayed or an advanced critical period of growth, to escape regularly recurrent periods of drought. It is important to remember that such plants cannot be considered as truly drough-resistant, since they would succumb in the event of an off-season drought. However, they are of great importance from an agricultural point of view in areas subjected to regular seasonal droughts.

Many phases of the drought resistance problem have been investigated with the hope of obtaining some relatively simple and practicable index for determining drought resistance. The relation of the root system to drought resistance has been the subject of considerable research. Earlier investigators in this field, Gain (16) and Hedgecock (18) believed that the relative drought resistance of plants might be determined by the degree to which they availed themselves of the soil moisture. This earlier belief was modified by Briggs and Shantz (10), who showed that different species of plants when grown on the same soil type showed little, if any, difference in "wilting coefficient". They did find that different soils varied considerably in this regard. Caldwell (13) noted further that the wilting coefficient varied with environmental conditions. Plants growing under humid or shady conditions could reduce the water content of the soil to a much greater degree than those grown under

dry or sunny conditions. Ivanov (20) has stressed the importance of the root system in relation to drought resistance. He points out that plants with roots of high suction power are in the most favorable position to resist drought. He believes the denseness of the root hairs to be an important characteristic of a resistant plant. It enables the plant to tap a much greater volume of soil than it would otherwise. He believes, furthermore, that these factors are important under conditions of high transpiration (atmospheric drought), when a rapid moisture supply is necessary to prevent wilting of the plant.

Talanov (43) reported that the drought resistance of Caesium and Milturum, two West Siberian wheats, was due in part to both a rapidly developed primary root system, and a profusely branched root system in its ultimate aspect. He states that "the root systems of these varieties touch the soil particles with a greater surface and absorb water from a greater volume of soil (up to the limiting minimum, connected with the peculiarities of the soil)." Miller (28) reported that the nature of the root system may play an important role in preventing incipient wilting—the drying out of the epidermal cells. The rate of transpiration of sorghum per unit of leaf area was found to be twice that of corn, while the leaf area was only 25% as great. An examination of the root systems of these two crops showed that, while both had the same number of roots of the first order, sorghum possessed twice as many roots of the second order. Miller believes that the greater number of finer roots of sorghum, together with the smaller leaf surface, are instrumental in keeping the water supply of the leaf sufficient to retard incipient wilting.

For a long time xerophytes were believed to have low transpiration rates. Maximov and his associates (26) have shown that, contrary to the former belief, xerophytes were characterized by high transpiration rates. Zalenski (26), Ivanov (20), and Wilson (49) have corroborated these findings.

Briggs and Shantz (11, 12) and Shantz and Piemeisel (40) were unable to confirm their original premises of a close association between the water requirement of plants and drought resistance. They found that millet, sorghums and corn have a lower water requirement than wheat, oats or barley. The drought-resistant grasses, Agropyrons and bromes, were found to have a very high water requirement; while rice and buckwheat, relatively drought-susceptible species, showed a moderately low water requirement. Maximov and Alexander (26) were also unable to show a direct relation between water requirement and suitability for arid areas. They did find that there was a tendency for plants that transpired intensively to use the water less efficiently. Tulaikov (26) concluded that the water requirement of different members of the same species was quite similar. The differences were too small to justify making selections on this basis. Kiesselbach (21) came to similar conclusions in regard to the value of water requirement in selecting drought resistant strains of corn. Richardson (36), working in Australia, also failed to establish a definite correlation between drought resistance and water requirement. He found that different varieties of wheat showed little

variation in water requirement when the latter was calculated on the basis of total dry weight. Greater differences were noted when water requirement was calculated on weight of grain only. Dillman (14) found small but significant differences in water requirement of different varieties of the same crop, but did not consider them large enough to be used as a basis of selection.

A great deal of literature (15, 19, 26, 37, 44, 50) is available concerning the morphological and anatomical peculiarities which distinguish typical xerophytes. This literature is too extensive to be reviewed in this paper. It should be explained, however, at this point that earlier investigators believed that a drought-resistant structure functioned only in restricting transpiration. Maximov (26) holds the view that the study of xeromorphic structures is only useful when an attempt is made to correlate them with some corresponding physiologic change which may be important in drought resistance.

A number of investigators have studied the feasibility of selecting drought-resistant varieties on the basis of xeromorphic structures that have been induced under drought conditions. This method of approach in regard to selection has been advocated by Maximov (26). Probably the first work to be reported along this line was that of Kolkunov (22). He studied stomatal size in a number of wheat varieties possessing varying degrees of drought resistance, and found the more resistant varieties to be characterized by small stomata. He also found that in humid years the yield of sugar beets and corn was directly proportional, and in dry years inversely proportional to the size of the plant cell. Kolkunov was, however, working under the assumption that transpiration as regulated by the stomata was a good criterion of drought resistance. Yakushkina and Vavilov (26) working with 17 pure lines of different varieties of oats, found no correlation between cell size and yield of crop under humid conditions. Later Kolkunov (26) selected four pure lines of Beloturka wheat differing in cell size, and grew them under different soil moisture contents. He found that under conditions of high soil moisture, the larger celled varieties gave the highest grain yields, while the reverse was true in the case of low soil moisture conditions. Vasiliev (46) obtained no relation between length of stomata and size of cells in wheat. Pavlov (33) reported that, in general, the more drought-resistant and early varieties of winter wheat were characterized by small stomata, but this condition was not apparent in spring wheat and oats. Alexander (5) found a correlation in oats between root and shoot length in the seedling stage and drought resistance. The more drought-resistant varieties produced a relatively smaller proportion of root and shoot at all soil moisture contents, in comparison with the less resistant varieties. Recently Bolsunov (9), Snoep (42) and Pavlov (33) have reported that an index of drought resistance may be obtained by studying the osmotic pressure of germinating seeds, as determined by salt or sugar solutions. Seeds selected for high osmotic pressure gave higher yields than unselected material. Bolsunov (9) found this increase in yield to hold true, under both humid and arid conditions.

Working with the viewpoint of Maximov (26), that the only true criterion of drought resistance is the ability of the plant to endure drought without injury, several Russian investigators have tested the ability of a number of crop plants to resist drought. Tumanov (44) used percentage plant survival as an index of drought resistance, when testing the reaction of eight varieties of wheat to endure a prolonged period of soil drought. He found that varieties exhibiting drought resistant properties in field tests had a survival of over 80%, whereas varieties not exhibiting these properties had a survival of 50% or less. Later Tumanov (45) studied the relative drought resistance of a number of crop plants, including oats, corn, sorghum and alfalfa. The percentage of dead leaves after eight days without available water was used as a measure of drought resistance. He concluded that percentage of dead leaves was not always a good measure of drought resistance, since the degree of resistance of separate organs varied within different plants. Kondo (23), in a study of the resistance of sunflowers and soybeans to soil drought, used both percentage of dead leaves and reduction in grain yield as criteria of drought resistance. Krasnosselsky-Maximov (24) also used these two measures of susceptibility when estimating the injury occurring to cereals as a result of exposure to artificial dry winds.

Physico-chemical studies regarding the nature of drought resistance is the most recent phase of the problem to receive attention. Newton and Martin (32) have studied this phase of the problem for a number of crop plants. Osmotic pressure of tissue fluids was found to vary with the physiological scarcity of water, but was not considered a good index of drought resistance. Bound water content of the cell sap gave more satisfactory results, for it was possible to class a number of cultivated wheats and grasses in order of their drought resistance. These authors suggest that it is the high colloidal content of the cells, which reduces the abstraction of water from them, that accounts for the lack of injury shown in drought-resistant plants. Vasiliev (47), who experimented with wheat plants, believes that sugars play an important role in the protection of plants from desiccation. As drought conditions increase in intensity, increasing amounts of starch are converted into sugar. When water conditions improve, the process is reversed, the soluble carbohydrates decreasing and the insoluble carbohydrates increasing in quantity.

Materials and Methods

Serving as standards of comparison, two Russian wheat varieties of reputed drought-resistant qualities, Milturum 0.321 and Caesium 0.111, introduced from Russia in 1928, have been included in all the studies reported in this paper. These varieties have also exhibited drought resistance in trials conducted at the University of Alberta.

The soil used throughout these investigations, unless otherwise stated, was a brown transitional type between the black and the gray soils obtained from Stony Plain, Alberta. This soil has the same physical properties as the brown soil that is typical of the drought areas of southern Alberta.

In all pot-work reported, six-inch porous clay pots were used. Five kernels per pot were sown. The individual plant was used as a basis of estimating drought injury. In interpreting the results, however, the average injury occurring to the plants in each treatment was used.

In the root studies reported, the root material was extricated from the soil mass by washing with a spray of water from the nozzle of a garden hose. After the root system had been carefully extricated it was floated on the surface of water to facilitate examination and collection on blotting paper and preservation, as shown in Fig. 5.

The studies on the reaction of various wheat varieties to atmospheric drought were made in a wind or "chinook" machine. This apparatus has been described in detail by Aamodt (1, 2). Atmospheric drought, similar to the conditions in the dry area of Alberta when growing grain is severely injured, was produced artificially in a glass tunnel. Wind was blown through the tunnel over the plants at a rate of approximately six miles per hour, at a temperature of 110° F., and a relative humidity of about 15%.

Experimental Results

1. Susceptibility of Wheat Varieties to Drought at Different Stages of Development

The purpose of this study was to determine the relative effects of soil and atmospheric drought on wheat varieties when exposed in the four growth stages, stooling, shooting, soft dough and hard dough. The effects of two soil types were also compared—a black chernozem soil, typical of the Edmonton district, and the transitional type. Two drought-resistant varieties, Milturum and Caesium, and two non-drought-resistant varieties, Marquis and Reward, were used in this study. The plants were grown in six-inch pots with optimum soil moisture conditions. Triplicate series of pots, with one series of three pots serving as a check, were used.

Plants subjected to the soil drought were deprived of water as they reached the stage of development selected for the test, and water was withheld until the plants showed evidence of extreme wilt. They were kept in this wilted condition for three days. Water was then applied to the soil to bring it up to the optimum moisture condition.

The plants subjected to atmospheric drought were given an eight hour exposure in the chinook machine at a temperature of 110° F. and with humidity ranging from 14–17%. The plants received no water during the eight hour exposure.

The percentage leaf loss of the four varieties tested for the stooling and shooting stages is given in Table I. It is necessary to explain at this point that, owing to natural maturation processes, the plants of all varieties when exposed to drought in the soft- and hard-dough stages lost all of their leaves. The phenomenon was similar to that occurring in naturally ripening grain, in that the straw tended to bleach and leaves became brown and desiccated. The leaves showed little evidence of true windburn injury. For this reason the data in Table I pertain only to the stooling and shooting stages.

The data on degree of shrivelling of the kernels resulting from exposure of plants to drought at the four stages of development are summarized in Table II. The degree of kernel shrivelling was estimated by placing the grain from each sample in one of 10 classes. Extremely shrivelled kernels were placed in Class 1, and plump kernels in Class 10. In the studies on breeding for drought resistance in spring wheat, a more accurate determination of kernel plumpness was desired than that usually obtained by taking weight per 1000 kernels. A method was developed for obtaining weight per measured bushel of grain from individual wheat plants (3). The degree of association between weight per measured bushel, obtained by the above method, and an estimated degree of kernel plumpness was very high.

The data given in Table I are based on the average injury shown by the five plants in each pot. The yield of grain from individual plants was too

TABLE I

THE COMPARATIVE RESISTANCE TO ATMOSPHERIC AND SOIL DROUGHT OF FOUR VARIETIES OF WHEAT GROWN IN BOTH BROWN AND BLACK SOILS WHEN EXPOSED AT THE STOOLING AND SHOOTING STAGES

Variety	Percentage leaf loss by							
	Atmospheric drought				Soil drought			
	Stooling stage		Shooting stage		Stooling stage		Shooting stage	
	Brown soil	Black soil	Brown soil	Black soil	Brown soil	Black soil	Brown soil	Black soil
Marquis	95	95	60	*100	5	5	70	40
	85	50	*100	*100	trace	5	50	40
	*100	*100	85	*100	trace	5	50	40
Reward	90	25	*100	95	trace	20–25	90	85
	50	25	*100	*100	10	20	85	40
	90	25	95	95	10	20–25	85	100
Milturum	50	20	*100	100	trace	10–15	50	20
	40	20	100	95	trace	10–15	40	20
	30	20	*100	95	5	10–15	40	20
Caesium	10	10	65	100	trace	20	60	40
	15	10	*100	100	15	10–15	60	35
	10	10	*100	100	15	10–15	75	20

Plants completely killed.

small to permit a reliable estimate to be made of the degree of kernel shrivelling. An estimate of the degree of kernel shrivelling was made on the bulked grain from all of the plants in each treatment. The data are given in Table II.

From a consideration of the data in Table I, it is evident that under the conditions of the experiment much greater injury to the plants resulted from atmospheric drought than from soil drought in the stooling and shooting stages. The effect of atmospheric and soil drought on kernel shrivelling was approximately the same in the stooling stage of development (Table II).

Atmospheric drought was far more severe than soil drought in the shooting stage, as is shown by the percentage of leaf loss (Table I) and the lack of kernel development (Table II).

TABLE II

AVERAGE DEGREE OF SHRIVELLING OF KERNELS OF FOUR VARIETIES OF WHEAT GROWN IN BROWN AND BLACK SOILS WHEN EXPOSED TO ATMOSPHERIC AND SOIL DROUGHT AT FOUR STAGES OF DEVELOPMENT

Variety	Average degree of kernel shrivelling*																	
	Check		Atmospheric drought								Soil drought							
			Stooling		Shooting		Soft dough		Hard dough		Stooling		Shooting**		Soft dough		Hard dough	
	Br.†	Bl.†	Br.	Bl.	Br.	Bl.	Br.	Bl.	Br.	Bl.	Br.	Bl.	Br.	Bl.	Br.	Bl.	Br.	Bl.
Marquis	9	10	9	9	7	0	2	3	6	9	10	10	10	8	2	2	10	5
Reward	9	9	9	9	0	0	8	9	8	9	9	8	9	6	2	6	9	6
Milturum	9	8	9	9	0	0	3	5	10	9	10	7	7	5	4	6	10	7
Cæsium	10	9	9	9	0	0	3	2	9	9	9	8	9	7	5	4	9	8

Scale of 1 = shrivelled to 10 = plump; 0 = no seed formed.
†*Br. = brown and Bl. = black soil.*
***Kernels produced largely from second growth.*

Exposure of plants to drought in the stooling stage resulted in injury of a temporary nature, as plants injured at this time made relatively rapid recovery and produced spikes bearing normal plump kernels. The rate of recovery was found to be roughly inversely proportional to the amount of leaf injury. Marquis, Reward and Milturum made a slower rate of recovery after exposure to atmospheric drought than after exposure to soil drought. Generally it was observed that, if foliage injury was not too severe, plants exposed in the stooling stage recovered sufficiently rapidly to mature only a few days later than the unexposed checks. Hence it is felt that plants injured by drought in the stooling stage under field conditions, and in areas of a moderately long growing season, will have considerable economic value, if favorable conditions prevail in the later stages of growth.

Much greater leaf loss resulted when plants were exposed to drought in the shooting stage. This was accompanied by greater culm injuries and a much slower rate of recovery. Culm injury was particularly severe in plants subjected in this stage to atmospheric drought, and resulted in the death of a considerable number of the plants and in almost complete sterility of those which survived. Plants exposed to soil drought in the shooting stage, while exhibiting comparatively high leaf loss, recovered sufficiently to produce culms bearing fairly plump kernels. However, it is necessary to point out in this connection that the greater part of the spikes reaching maturity were produced from second growth, and were much later in maturing than those of the check plants, or those of plants subjected to atmospheric drought during the stooling stage. It is obvious that plants forced to produce new culms after having once reached the shooting stage would have little economic value.

Great reduction in kernel yield due to shrivelling occurred when plants were subjected to drought (either soil or atmospheric) in the soft-dough stage; whereas, after the kernels had reached the hard-dough stage, they were only slightly affected by drought.

The effect of soil type on the degree of plant injury arising from drought was different in the different stages of development. Plants grown in brown soil showed greater consistent leaf losses than those grown in black soil, when exposed to atmospheric drought during the stooling stage and to soil drought in the shooting stage (Table I). The reverse was true when plants were subjected to soil drought during the stooling stage.

2. Hardening of Wheat Plants as a Means of Increasing Their Resistance to Atmospheric Drought

Many plants have the inherent ability to adjust themselves physiologically to adverse conditions when exposed gradually to unfavorable factors. This phenomenon is well illustrated by the work that has been done on the testing of varieties of winter wheat for resistance to low temperatures. The inherent potentialities of varieties to withstand injury from exposure to low temperatures are more clearly differentiated after a "hardening-off" process than when not so treated. General observations and reports in the literature indicated that limited exposures of plants to drought developed a greater degree of tolerance to more severe exposures later on. Studies of the influence of the hardening process on varietal reaction to atmospheric drought were carried out, using the two drought-resistant varieties, Milturum and Caesium, and the two susceptible ones, Marquis and Reward. In the first experiment the plants were grown in wooden boxes holding approximately one cubic foot of soil. The boxes were placed outdoors, where the plants were exposed to the natural atmospheric conditions. Two rows of plants were grown in each box, the rate of seeding being equivalent to one and one-half bushels per acre. Each treatment was carried out in duplicate.

The hardening process consisted in allowing the soil in the boxes to dry out until the plants showed signs of severe wilting, *i.e.*, cell injury of the leaves just beginning to be apparent. The moisture in the soil at this time was considerably below 20% of the water holding capacity. Moisture was then added to the soil and the plants were allowed three days to regain their normal condition. They were then given an eight hour exposure in the "chinook" machine at 110° F., 16% relative humidity and a wind velocity of six miles per hour. At the time of the exposure to windburn, Milturum and Caesium were in the late stooling stage, while Marquis and Reward had just commenced to shoot. The check plants, which had received optimum moisture at all times and consequently were not hardened, were given a similar exposure.

The results of this first experiment are given in Table III. They show clearly the greater resistance to windburn of hardened plants of all four varieties as compared with those not receiving the preliminary treatment.

One important feature of the data in Table III is the high percentage of culm injury shown by the non-hardened plants of Marquis, Reward and Caesium. Culm injury is obviously a very serious matter, as plants injured in this way make slow, if any, recovery. In this experiment no recovery occurred among the non-hardened plants of Reward. Milturum, on the other hand, made comparatively rapid recovery; whereas Marquis and Caesium

TABLE III

LEAF AND CULM INJURY TO FOUR VARIETIES OF WHEAT WHEN EXPOSED TO ATMOSPHERIC DROUGHT WITH AND WITHOUT PREVIOUS HARDENING

Variety	Stage of growth	Degree of injury			
		Not hardened		Hardened	
		% leaf loss	% culm loss	% leaf loss	% culm loss
Marquis	Early shooting	95	100	40	0
		85	40	25	0
Reward	Shooting	100	100	10 + 50 chlorosis	0
		100	100		
Milturum	Late stooling	20 + 50 chlorosis	0	trace	0
		15 + 15 chlorosis	0	trace	0
Caesium	Late stooling to early shooting	75	25	20	0
		85	75	60 chlorosis only	0

were intermediate. The injured plants of the hardened lots made rapid recovery in all cases, undoubtedly because of lack of serious culm injury. A number of leaves were found to exhibit chlorosis. When this condition was severe, death of the leaf followed, and when mild, the leaves recovered and appeared normal. Thus a considerable number of the leaves on non-hardened Milturum plants, as well as on the hardened Reward plants, eventually died. Fig. 1 shows a comparison of the hardened and non-hardened plants of Reward and Milturum approximately one month after exposure.

As a result of culm injury, a difficulty arises in assigning injury percentages to the leaves. It is evident that the death of a culm results in death of all the leaves associated with it. However, notations made on leaf injury a few days after exposure to the hot wind often show only a portion of the leaf to be actually injured. Since a period of two to three weeks is necessary before death of the culm is complete, the question arises whether percentage leaf loss should be limited to that arising directly from leaf burn, or whether that associated with death of the culms should also be included. In the present study leaf injury was estimated a few days following exposure; or, in other words, it was calculated independently of loss due to death of the culms.

FIG. 1. *Effects of hardening, by soil drought, on the ability of two wheat varieties to withstand atmospheric drought. Left to right: Reward—hardened; Reward—non-hardened; Milturum—hardened; Milturum—non-hardened. (Photograph taken 1 month after exposure).*

The data in Table IV summarize the results of a second experiment, in which Marquis and Milturum were tested in a manner similar to that described above. The plants of these varieties were in the late stooling to early shooting

TABLE IV

PERCENTAGE OF LEAF INJURY TO TWO VARIETIES OF WHEAT WHEN EXPOSED TO ATMOSPHERIC DROUGHT, WITH AND WITHOUT PREVIOUS HARDENING

Variety	Stage of growth	Leaf loss due to soil drought, %	Leaf loss due to atmospheric drought	
			Non-hardened, %	Hardened, %
Marquis	Early shooting	15	50	20
		15	25	30
Milturum	Late stooling	5	85	0
		15	60	5

stages. It will be noted that a certain amount of injury resulted directly from the hardening process. The difference in leaf loss between the hardened and unhardened plants of Marquis is not so striking as in the former experiment. However, that manifested by Milturum is even greater. Little evidence of culm injury was observed in this experiment. The comparatively heavy leaf loss shown by the unhardened plants of Milturum can be attributed only to experimental variation.

The boxes used in the above two experiments were difficult to handle in the chinook machine. Also the number was necessarily limited because of

the size. Consequently it was thought advisable to study the effects of hardening plants grown in ordinary six-inch pots. Marquis and Milturum were selected for the experiment. Ten pots of five plants each were used in each treatment. The plan of the experiment was as follows:

1. Check plants received an eight hour exposure in the chinook machine without previous hardening.

2. Plants hardened by two consecutive exposures of three and a half hours each in the chinook machine, followed by a final exposure of eight hours.

3. Plants hardened by exposure to soil drought (water holding capacity approximately 15%), followed by an eight hour exposure in the chinook machine.

After each hardening treatment, the plants were watered and allowed sufficient time to recover to approximate normality. Owing to considerable variation among plants within each treatment, the average percentage leaf loss occurring in the plants contained in each series of 10 pots was used to interpret the results.

The results of this experiment, as shown by the data in Table V, again illustrate the relative resistance to windburn of hardened plants as compared with those not receiving this treatment. The least leaf loss occurred in the

TABLE V

PERCENTAGE LEAF LOSS OF TWO VARIETIES OF WHEAT GROWN IN POTS AND EXPOSED TO ATMOSPHERIC DROUGHT FOR EIGHT HOURS WHEN NON-HARDENED, WHEN HARDENED BY LIMITED EXPOSURES TO ATMOSPHERIC DROUGHT, AND WHEN HARDENED BY SOIL DROUGHT

Variety	Check		Hardened by atmospheric drought		Hardened by soil drought	
	Leaf loss, %	Leaves chlorotic, %	Leaf loss, %	Leaves chlorotic, %	Leaf loss, %	Leaves chlorotic, %
Marquis	80	15	30	trace	9	0
Milturum	35	22	12	trace	11	0

plants hardened by exposure to soil drought. The plants hardened by short exposures to atmospheric drought showed little leaf wilt after the first hardening exposure, but exhibited considerable chlorosis. The combined percentages of leaf wilt and chlorosis very closely approximated the total leaf injury noted after the final eight hour exposure. This indicates that very little additional injury occurred to the leaves following this initial treatment. A greater leaf loss at the termination of the experiment was due to a more thorough wilting of the already chlorotic leaves. There was no additional injury to the leaves after the second hardening exposure. This may be explained by assuming that the duration of the exposure at that time was not sufficiently long to bring about further leaf injury. Fig. 2 shows the degree of injury occurring

in Marquis plants hardened by short exposures in the chinook machine, when given a severe exposure to atmospheric drought, compared with plants not hardened.

FIG. 2. *Effects of hardening, by short exposures to atmospheric drought, on the ability of Marquis plants to withstand a long exposure to atmospheric drought. Four pots on left, hardened. Four pots on right, non-hardened.*

FIG. 3. *A comparison of the ability of two varieties of wheat to recover from exposures to soil drought. Two pots on left, Reward; two pots on right, Caesium. (Photograph taken two weeks after rewatering).*

Reward was included in one of the experiments in which the plants were to be hardened by soil drought previous to exposures to atmospheric drought. It appeared to be especially susceptible to soil drought as compared with Caesium as is shown by the following observation. Plants of Reward and Caesium were grown in pots in which the soil moisture was allowed to reach a point which caused the plants to wilt. When the moisture was again added to the soil, Caesium made rapid recovery and soon showed normal leaf development. Reward failed to recover and eventually died. The appearance of the two varieties two weeks after re-watering is shown in Fig. 3.

Methods 3. Varietal Reaction to Atmospheric Drought

The varieties selected for a preliminary study of varietal reaction to artificially produced drought are as follows: Baart and Federation, two soft wheats, reported as exhibiting qualities of drought resistance in the United States and Australia; Pentad and Kubanka, two durum wheats, included in the study owing to certain conflicting reports concerning the drought-resisting properties of durum wheats; Milturum, a drought-resistant Russian wheat; Canus, a promising new variety developed at the University of Alberta; Red Bobs No. 222, a relatively early, high yielding, common wheat.

In experiments with these seven varieties, each variety was replicated three times in six-inch pots. All exposures in the chinook machine were made after the plants had reached either the late stooling or early shooting stages. The temperature prevailing during the exposures was 110° F., the relative humidity varied from 13–18%, and the wind velocity from six to seven miles per hour.

In the first experiment the plants were exposed for eight hours in the chinook machine, without previous hardening. After this treatment the plants were allowed five days to recover with an optimum soil moisture content. They were then re-exposed for 15 hours. After each exposure the percentage of leaves killed was estimated and recorded; also the percentage of plants killed and the percentage of culms killed on the plants that survived. The data are given in Table VI. These represent averages based on the total number of plants of each variety used per treatment.

In the second experiment the plants were hardened by permitting them to wilt temporarily as a result of soil drought. After this preliminary hardening process the plants were allowed to recover, and they were then exposed to atmospheric drought in exactly the same manner as the plants in the first experiment. After a period of two weeks the plants were given a third exposure to atmospheric drought for 15 hours. The injury to the plants was estimated as in the first experiment. When the plants began to recover after the third exposure a number of new shoots developed. Notes on new shoot development were also recorded. The data obtained in this experiment are summarized in Table VII.

In the third experiment, the plants were given a single exposure of 15 hours to atmospheric drought, without previous hardening. The data obtained are given in Table VIII.

The modifications in the manner of conducting these experiments were made in an attempt to parallel as closely as possible natural occurrences in the field. Under natural conditions, plants are seldom subjected to sudden periods of hot dry winds, but are usually "hardened off" by gradually increasing temperatures, or by limited soil moisture. Similarly, they are seldom exposed to a single period of hot wind, but usually have to weather several hot periods in a single growing season.

Results

The results of the first experiment, summarized in Table VI, show the varieties tested to fall into three groups as regards susceptibility to atmospheric drought. The first of these, comprising the susceptible varieties, includes the

TABLE VI

COMPARATIVE INJURY TO SEVEN VARIETIES OF WHEAT AFTER TWO EXPOSURES TO ATMOSPHERIC DROUGHT WITHOUT PRELIMINARY HARDENING OF THE PLANTS

Variety	Total number of plants	Total leaf loss in per cent after—		Plants killed, %	Culms killed, %
		First exposure (8 hr.)	Second exposure (15 hr.)		
Federation	15	47	65	27	42
Baart	15	25	80	73	77
Red Bobs No. 222	17	5	62	12	43
Kubanka	15	15	100	100	100
Pentad	15	10	88	47	57
Canus	16	trace	15	0	0
Milturum	16	trace	20	5	0

two durums Kubanka and Pentad, and the soft wheat Baart. Of these, Kubanka shows the greatest injury, with 100% leaf, culm and plant loss. Pentad and Baart exhibit an equal leaf loss of approximately 80%, but differ somewhat in the percentage of dead culms and plants. Baart shows the greater injury in these respects. Federation and Red Bobs No. 222 fall into a second group, and may be termed moderately susceptible. The varieties exhibited approximately 60% loss of leaf and 40% of culm. Federation showed a greater number of dead plants than Red Bobs No. 222. The resistant group, consisting of Milturum and Canus, exhibited less than 25% leaf loss and little injury of the culms.

It will be evident that the ranking of the varieties with regard to percentage leaf loss, following the initial exposure of eight hours, is not in direct agreement with that shown after two successive exposures. Kubanka and Pentad show comparatively less injury and Federation comparatively more after the first

exposure than they do after the two successive exposures. Evidently a single exposure of eight hours is not sufficient to bring out true varietal differences.

The results of the second experiment (Table VII) show up essentially the same varietal differences as the first. The injury exhibited by the plants in this experiment is not as severe as in the former, no doubt owing to the initial hardening treatment given the plants. A certain amount of re-stooling of the plants occurred following the three consecutive exposures. Baart, Red Bobs No. 222 and Milturum showed the greatest tendency in this regard.

TABLE VII

COMPARATIVE INJURY TO SEVEN VARIETIES OF WHEAT AFTER PRELIMINARY HARDENING AND EXPOSURES TO ATMOSPHERIC DROUGHT

Variety	Total number of plants	Total leaf loss in per cent shown after—			Plants killed, %	Culms killed, %	Average number of new shoots per plant
		First exposure (8 hr.)	Second exposure (15 hr.)	Third exposure 15(hr.)			
Federation	14	trace	22	32	8	10	0.8
Baart	15	trace	23	37	0	23	1.5
Red Bobs No. 222	14	trace	20	30	0	0	1.2
Kubanka	15	5	37	60	33	38	0
Pentad	15	5	27	58	33	42	0.3
Canus	15	trace	17	25	0	5	0.3
Milturum	15	trace	7	15	0	0	1.3

With the exception of Red Bobs No. 222, which showed comparatively greater injury, the behavior of the varieties to a single exposure of 15 hours, (third experiment, summarized in Table VIII) was in general accord with those of the two former experiments. No explanation can be given other than that Red Bobs is particularly susceptible to long exposures in the unhardened condition. It will be noted that no culm loss occurred in the case of the varieties Canus and Milturum, while the smallest loss in the other varieties was 22%.

TABLE VIII

COMPARATIVE INJURY TO SEVEN VARIETIES OF WHEAT AFTER A FIFTEEN HOUR EXPOSURE TO ATMOSPHERIC DROUGHT WITHOUT PRELIMINARY HARDENING OF THE PLANTS

Variety	Total number of plants	Loss in per cent		Plants killed, %	Average number of new shoots per plant
		Leaves	Culms		
Federation	15	37	22	0	0.1
Baart	17	45	37	0	0.3
Red Bobs No. 222	11	55	43	18	1.0
Kubanka	15	75	43	40	0
Pentad	15	90	83	80	0
Canus	15	25	0	0	1.0
Milturum	15	20	0	0	1.3

Consistent specific and varietal differences in regard to resistance to windburn injury are apparent from the results of these three experiments. The two durum varieties Pentad and Kubanka, and the soft wheat Baart, appeared particularly susceptible, as judged by leaf, culm and plant loss. Federation and Red Bobs No. 222 evinced susceptibility but were not as severely injured as the former. Milturum and Canus, on the other hand, showed considerable resistance (See Fig. 4). These varieties exhibited comparatively small percentages of leaf and culm loss and, for this reason, showed rapid recovery following exposure to drought.

FIG. 4. *Reaction of six wheat varieties following two successive exposures of 8 and 15 hours to atmospheric drought. No previous hardening. Back row, left to right;—Red Bobs No. 222, Canus, Milturum. Front row, left to right;—Kubanka, Baart, Pentad.*

There was fairly good agreement between the different measures of injury used. Thus the varieties Pentad, Kubanka and Baart, which ranked highest in leaf loss, also ranked highest in per cent dead culms and per cent dead plants. On the other hand the low leaf loss exhibited by Canus and Milturum was accompanied by a correspondingly low loss of culms.

General observations which could not be readily expressed in tabular form suggested that there were two aspects of the resistance shown by Milturum. There was an inherent resistance of the cells to desiccation and a remarkable capacity on the part of the plant to produce new leaves and shoots. Canus, while not showing any marked capacity to produce new leaves, showed a high resistance to desiccation which allowed the plants to develop uninterrupted by exposure to the hot winds. The surviving plants of the other varieties tested, particularly those of Pentad and Kubanka, often failed to regain normal vigor. In many cases only one culm reached maturity. Many

of these mature culms showed abnormalities, being so bent at the nodes as to be almost recumbent.

In view of the great susceptibility to windburn shown by the two durum varieties, the findings of certain Russian investigators are interesting (34). Varieties of *T. durum* were found to be characterized by considerable drought resistance during the period of ripening, but did not show this characteristic in the initial growth stages. The present work shows the extreme susceptibility of durums to atmospheric drought in the earlier stages.

The beneficial effects of hardening the plants as a means of rendering them more tolerant to the effect of atmospheric drought is clearly evident from a comparison of the data contained in Table VI (compiled from unhardened plants), and those in Table VII (compiled from hardened plants). Much greater leaf loss occurred after comparable exposures when the plants were unhardened than when they were hardened. The hardened plants also exhibited a considerably lower percentage of dead culms and plants.

4. Studies on Primary Root Development of Wheat Varieties

Varietal Differences

Talanov (43) states that one of the important contributory factors to the drought resistance possessed by Milturum and Caesium lies in the character of their root systems. These varieties have the capacity to develop their root systems rapidly during the early stages of growth before full tillering. The root systems are also greater in their ultimate aspect in that they are profusely branched. Talanov believes that the root systems of these varieties absorb water from a greater volume of soil (up to the limiting minimum connected with the peculiarites of the soil).

In view of Talanov's work, it was decided to commence a study of the early root development of some of the varieties commonly grown in western Canada, and also of certain drought-resistant varieties. In a preliminary experiment conducted during the summer of 1933 root studies were made of Milturum, Caesium, Reward and Marquis. The plants were grown in boxes (1 cu. ft.) placed outdoors. Two rows of 35 seeds (rate equivalent to one and one-half bushels per acre) were sown per box. There were four replicates of each variety, two to be examined for root development at the end of two weeks, and two at the end of one month.

The first examination of the root systems was made 16 days after emergence, when the plants possessed from five to seven leaves. It must be remembered that at this time no secondary roots were evident, and hence all discussion to follow is concerned only with primary roots. Distinct varietal differences in density of root system were apparent. Milturum and Caesium had developed a much more highly branched system than was evident in Marquis and Reward. The root system of Reward seemed especially lacking in this respect. General observations indicated that, while no great varietal differences were apparent in the number of primary roots produced, the drought-resistant varieties had a slightly larger average number. No differences

existed between varieties in length of root systems, all having reached a length of 12 to 14 inches. For purposes of photography the root systems of 25 random plants were floated on to cardboard, and teased out to form a mat three and a half inches in width. Fig. 5 shows the development of the root systems of the four varieties in the test, 16 days after emergence.

FIG. 5. *Primary root systems of seedlings of drought-resistant and non-drought-resistant varieties of wheat, sixteen days after emergence. Left to right—Milturum, Caesium (drought-resistant), Marquis, Reward (non-drought-resistant).*

Another examination of the roots was made 31 days after emergence. At this time, the secondary roots had made their appearance and were extensive enough to complicate the examination of the primary roots. Furthermore, the primary roots had outgrown their boxes and were badly matted at the bottom, making it difficult to disentangle them. It was quite apparent, however, that the differences noted after two weeks of growth still persisted.

The results of this preliminary experiment were so interesting that arrangements were made to continue these studies in the greenhouse during the winter of 1933–34. For this work, special boxes were constructed, with two removable sides to facilitate the washing out of the roots from the soil. These boxes measured 10 × 10 × 9 in. and contained a little more than one-half a cubic foot of soil. Two rows of 25 seeds were sown per box, and all studies were carried out in duplicate. The varieties used in this study were Reward.

Garnet, Red Bobs No. 222, Marquis, Reliance and Canus; two soft wheats, Baart and Federation; two durums, Pentad and Pelissier; and two with drought-resistant properties, Milturum and Caesium. Wild oats were also included in this study.

Before discussing the results of this experiment, it is necessary to make some comment on the unsuitable growing conditions prevailing during the months of November and December, the period in which the plants were growing. During this interval of time the amount of sunlight was greatly limited by cloudy weather and by low outdoor temperatures that kept the glass covered with frost. This resulted in spindly, weak plants, lacking the healthy green color of normal seedlings. At the end of 23 days following emergence, little stooling of the plants was evident and only three or four leaves had formed. Root studies made at this time soon revealed the fact that root development had also been adversely affected. The development was scarcely more than half of that shown by plants grown outdoors in the summer. The method of study as originally planned consisted in recording the number of primary roots produced by each plant, and in devising a scale for estimating the degree of branching of these roots. It was found impossible, however, to do more than simply estimate the degree of branching, owing to the great variation between plants in the same box. This variation appeared to be due to the caking of the soil, which checked the normal growth of the roots downward, resulting in a well branched, but short, root system.

A summary of the results obtained in this study is to be found in Table IX. The data are expressed as an average for the total number of plants in each treatment. Distinct varietal differences in the number of primary roots developed are apparent. Under the conditions of the experiment Pelissier and Pentad, the two durums, distinctly excelled in this regard, having an average of practically five primary roots per plant. Milturum, Caesium and Baart had an average of over four roots per plant, while Marquis, Red Bobs No. 222 and Garnet, the next three varieties of merit, all had an average of only slightly over 3.6 roots per plant. Reward, Federation and Canus average only slightly over three roots per plant. Of the wheat varieties tested, Reliance shows the smallest number of primary roots, averaging only three roots

TABLE IX

COMPARATIVE PRIMARY ROOT DEVELOPMENT OF SEEDLINGS OF TWELVE VARIETIES OF WHEAT AND WILD OATS TWENTY-THREE DAYS AFTER EMERGENCE

Variety	No. of primary roots per 100 plants	Branching
Pelissier	499	good
Pentad	485	good
Milturum	441	good
Caesium	406	good
Baart	402	poor
Red Bobs No. 222	365	fair
Garnet	364	fair
Marquis	362	fair
Reward	341	poor
Federation	333	poor
Canus	321	fair
Reliance	304	excellent
Wild oats	300	poor

per plant. The root system of this variety, however, excelled all others in the degree of branching. It will be noted that of the other varieties only Milturum, Caesium and the durums showed well branched root systems. Baart, Reward, Federation and wild oats were distinctly lacking in this regard; while Marquis, Red Bobs No. 222, Garnet and Canus can be classed only as fair. Wild oats averaged only three primary roots per plant.

In view of the unfavorable growing conditions prevailing during this experiment, it would seem advisable not to attach too much significance to the above data. However, from results of a later experiment, carried out under more favorable conditions (reported in the next section of this paper), it was found that the branching of the roots was handicapped to a much greater degree than was the actual capacity to produce primary roots. Hence it may be concluded that, from the point of view of primary root development, the varieties are listed in Table IX in approximately the correct order. Further work in regard to varietal differences in the degree of branching of the roots is necessary.

Effect of Phosphorus on Primary Root Development

The general plan of this experiment differed from the one above, in that only the four varieties Reward, Marquis, Milturum and Caesium were used, and that superphosphate was added to the soil of one series of boxes. The rate was approximately 300 lb. per acre. Growing conditions in the greenhouse had improved greatly in the meantime, which was reflected in increased vigor of the seedlings. There was a very close agreement in the number of primary roots in the duplicates. The effect of phosphorus on the development of primary roots is shown by the data in Table X.

It is evident that with the exception of Reward, the addition of phosphorus did not stimulate primary root production, but, on the contrary, seemed to check such a tendency. A comparison of the primary root development of the varieties Marquis, Milturum and Caesium from untreated boxes, as shown in Table X, with that shown by the same varieties in Table IX, revealed little difference in number of primary roots. Evidently the growing conditions in the two experiments had little influence on the formation of the number of primary roots produced by these varieties. A similar comparison in the case of Reward indicates that the growing conditions prevailing in this experiment had some

TABLE X

EFFECT OF SUPERPHOSPHATE ON PRIMARY ROOT DEVELOPMENT OF WHEAT SEEDLINGS

Variety	Treatment	Number of primary roots per 100 plants			Increase or decrease of treatment over check
		1	2	Ave.	
Reward	P	420	448	434	
	No P	406	411	409	+25
Marquis	P	332	328	330	
	No P	340	336	338	− 8
Milturum	P	450	440	445	
	No P	458	466	462	−17
Caesium	P	407	421	414	
	No P	433	445	439	−25

effect on the primary root formation of this variety. Under these conditions there appeared to be a slight beneficial effect from phosphorus on the development of primary roots in Reward.

A rough approximation of degree of branching of the roots washed out from phosphorus-treated and untreated soil was obtained by floating the roots of 30 random plants from each treatment on the cardboard, where they could be examined closely. No stimulatory action of the phosphorus on root branching was observed.

5. Germination of Wheat in Various Concentrations of Sodium Chloride, Potassium Chloride and Sucrose

Recently a number of investigators have reported that it is possible to select drought resistant strains of different crops by the osmotic pressure exhibited by their germinating seed, as differentiated by salt and sugar concentrations. In order to test the feasibility of this method of determining drought resistance, the kernels of a number of wheat varieties, including drought-resistant and non-drought-resistant types, were germinated in varying concentrations of sugar and salt solutions. For this purpose a germinating tray was constructed by stretching a double layer of cheesecloth over a 10 × 6 × 1 in. wooden frame. The tray was divided by means of tin strips into 12 compartments. A different lot of seed was placed in each compartment. All portions of the tray or vessel likely to rust were given a liberal coating of "asphalt black" paint. The whole tray was then placed in a vessel containing the desired solution.

Experiments were conducted using different concentrations of sodium chloride, potassium chloride and sucrose. Distilled water was used as a check. To keep evaporation at a minimum all germination tests were carried out over water in a galvanized iron humidity chamber. As a further precautionary measure to insure constant concentrations, a change of solution was made after 72 hours. In addition to the two drought-resistant varieties Milturum and Caesium, tests were carried out with Reward, Garnet, Red Bobs No. 222 and Marquis. Two lots of 50 seeds each were used in every test.

The data obtained are given in Tables XI, XII and XIII. It will be seen from a study of the germination percentages in the check solutions that the kernels of Milturum, Caesium and Garnet naturally germinate more rapidly than those of the other three varieties. This rapid germination is apparent in all the concentrations of the chemicals used, but does not appear to increase with increased concentration. In other words, when the concentration of a given chemical is increased the germination of all six varieties is retarded somewhat, but the relative rapidity of germination of the varieties remains

the same. Hence, in the light of these experiments, the drought-resistant properties of Caesium and Milturum cannot be said to be necessarily associated with superior abilities of their kernels to germinate in the more concentrated solutions. It is possible, however, that this superior energy of germination is one of many characteristics of drought resistant varieties. If this be the situation, then Garnet, which cannot be classed as truly drought-resistant, must lack certain other essential properties necessary for drought resistance. It is interesting in this connection to note that Bolsunov (9) found that kernels from wheat strains selected for their ability to germinate in salt concentrations of 10 to 15 atmospheres showed an increased energy of germination, as compared with those from unselected strains.

TABLE XI

A COMPARISON OF GERMINATION PERCENTAGES OF SIX VARIETIES OF WHEAT WHEN TESTED IN VARIOUS CONCENTRATIONS OF SUCROSE ($C_{12}H_{22}O_{11}$)

Concentration of solution	Variety	%Germination at the end of									
		12 hr.	24 hr.	48 hr.	60 hr.	72 hr.	84 hr.	96 hr.	108 hr.	120 hr.	144 hr.
Check—distilled water	Reward	0	0	3	33	58	92	98	—	—	—
.1 *N*		0	0	1	6	18	69	91	98	—	—
.2 *N*		0	0	0	8	20	62	88	91	—	—
.25 *N*		0	0	0	2	15	63	80	86	91	—
.3 *N*		0	0	0	4	15	29	49	85	95	—
Check—distilled water	Marquis	0	0	1	35	66	87	96	—	—	—
.1 *N*		0	0	0	21	40	90	96	98	—	—
.2 *N*		0	0	0	7	36	68	91	94	—	—
.25 *N*		0	0	0	2	19	44	83	90	92	—
.3 *N*		0	0	0	3	14	41	70	85	88	—
Check—distilled water	Red Bobs 222	0	0	1	24	58	96	97	—	—	—
.1 *N*		0	0	1	15	33	81	98	100	—	—
.2 *N*		0	0	0	16	29	71	94	96	—	—
.25 *N*		0	0	0	4	18	39	69	95	97	—
.3 *N*		0	0	0	4	8	23	31	82	98	—
Check—distilled water	Garnet	0	2	6	67	80	98	—	—	—	—
.1 *N*		0	0	1	26	53	95	99	—	—	—
.2 *N*		0	0	0	23	66	93	96	—	—	—
.25 *N*		0	0	0	16	35	72	87	94	95	—
.3 *N*		0	0	0	13	19	56	67	94	97	—
Check—distilled water	Milturum	0	2	7	47	78	96	98	—	—	—
.1 *N*		0	1	3	31	60	94	96	97	—	—
.2 *N*		0	0	0	31	52	89	98	—	—	—
.25 *N*		0	0	0	24	34	71	88	93	96	—
.3 *N*		0	0	0	14	26	52	78	89	94	—
Check—distilled water	Caesium	0	3	7	48	75	100	—	—	—	—
.1 *N*		0	2	7	20	58	97	99	—	—	—
.2 *N*		0	0	0	24	49	86	95	99	—	—
.25 *N*		0	0	0	16	36	76	92	96	98	—
.3 *N*		0	0	0	6	21	39	52	77	94	—

It must be remembered that only "one salt" solutions were used in the present work. For this reason seedlings were unable to develop after the necessary growth elements contained in the kernels were used up. It would seem that a much more promising method of attacking this problem would be to make up balanced solutions of varying osmotic pressures. In this way not only could the germination rates be studied, but the seedlings could be actually grown in these solutions. This would afford the investigator a much greater opportunity of studying the value of osmotic pressures as an aid in the selection of drought resistant strains.

A number of investigators (9, 42) have reported good results in the selection of drought resistant strains of various crops, through the use of salt solutions. It is possible that these investigators were selecting from a heterogeneous population of a given variety, and were not concerned with varietal differences as a whole.

TABLE XII

A COMPARISON OF THE GERMINATION PERCENTAGES OF SIX VARIETIES OF WHEAT WHEN TESTED IN VARIOUS CONCENTRATIONS OF POTASSIUM CHLORIDE (KCl)

Concentration of solution	Variety	% germination at the end of							
		12 hr.	24 hr.	36 hr.	48 hr.	60 hr.	72 hr.	84 hr.	96 hr.
Check—distilled water	Reward	0	0	69	82	96	—	—	—
.2 *N*		0	0	4	35	64	69	70	—
.4 *N*		0	0	0	0	13	19	33	..
.6 *N*		0	0	0	0	0	0	0	—
Check—distilled water	Marquis	0	1	82	84	94	—	—	—
.2 *N*		0	0	1	10	47	61	77	—
.4 *N*		0	0	0	0	12	22	27	—
.6 *N*		0	0	0	0	0	0	0	—
Check—distilled water	Red Bobs 222	0	0	91	97	—	—	—	—
.2 *N*		0	0	4	22	59	79	94	—
.4 *N*		0	0	0	0	17	21	45	—
.6 *N*		0	0	0	0	0	0	0	—
Check—distilled water	Garnet	0	7	73	93	95	—	—	—
.2 *N*		0	3	23	71	84	84	89	—
.4 *N*		0	0	0	1	21	30	38	—
.6 *N*		0	0	0	0	0	0	0	—
Check—distilled water	Milturum	0	10	79	95	—	—	—	—
.2 *N*		0	4	12	32	68	75	80	—
.4 *N*		0	0	1	4	18	29	38	—
.6 *N*		0	0	0	0	0	0	2	—
Check—distilled water	Caesium	0	12	89	96	—	—	—	—
.2 *N*		0	7	25	60	81	81	84	—
.4 *N*		0	0	1	6	28	38	50	—
.6 *N*		0	0	0	0	0	0	2	—

TABLE XIII

A COMPARISION OF GERMINATION PERCENTAGES OF SIX VARIETIES OF WHEAT WHEN TESTED IN VARIOUS CONCENTRATIONS OF COMMON SALT (NaCl)

Concentration of solution	Variety	% germination at the end of								
		12 hr.	24 hr.	36 hr.	48 hr.	72 hr.	96 hr.	120 hr.	144 hr.	168 hr.
Check—distilled water	Reward	0	1	32	94	95	—	—	—	—
.1 *N*		0	2	54	100	—	—	—	—	—
.2 *N*		0	0	10	24	75	89	—	—	—
.3 *N*		0	0	0	6	47	83	93	97	—
.4 *N*		0	0	0	1	4	23	66	81	—
Check—distilled water	Marquis	0	2	28	96	99	—	—	—	—
.1 *N*		0	0	70	97	—	—	—	—	—
.2 *N*		0	0	18	36	86	93	—	—	—
.3 *N*		0	0	0	4	61	86	93	—	—
.4 *N*		0	0	0	1	24	64	76	79	—
Check—distilled water	Red Bobs 222	0	5	31	96	99	—	—	—	—
.1 *N*		0	2	65	95	—	—	—	—	—
.2 *N*		0	1	20	42	95	99	—	—	—
.3 *N*		0	0	0	6	55	91	96	—	—
.4 *N*		0	0	0	5	35	66	89	—	—
Check—distilled water	Garnet	0	9	41	89	96	—	—	—	—
.1 *N*		0	8	96	—	—	—	—	—	—
.2 *N*		0	0	42	64	94	—	—	—	—
.3 *N*		0	0	0	18	86	95	—	—	—
.4 *N*		0	0	0	8	67	88	92	—	—
Check—distilled water	Milturum	0	14	75	96	—	—	—	—	—
.1 *N*		0	0	92	99	—	—	—	—	—
.2 *N*		0	4	38	58	90	98	—	—	—
.3 *N*		0	0	0	15	73	91	93	—	—
.4 *N*		0	0	0	13	69	82	84	—	—
Check—distilled water	Caesium	0	14	73	98	—	—	—	—	—
.1 *N*		0	4	83	97	—	—	—	—	—
.2 *N*		0	4	37	50	88	97	—	—	—
.3 *N*		0	0	0	17	67	97	—	—	—
.4 *N*		0	0	0	8	58	78	85	—	—

Conclusions

The work reported in this paper summarizes a number of attempts to obtain information regarding the nature of drought resistance, particularly as it exists in wheat. The method of attack consisted, in general, in comparing certain physiological and morphological features of the drought-resistant Russian varieties Milturum and Caesium with those of commonly grown susceptible types. These studies have indicated that at least three factors contribute to the drought resistance of these Russian varieties.

In the first place, these varieties have, in contrast to Reward, Marquis, etc., the ability to evade early periods of drought. This characteristic is particularly evident in the case of Milturum. It has been shown in this paper that these Russian varieties possess greater resistance to atmospheric drought

than the commonly grown types, when tested in the stooling stage. This superior resistance is not evident at the critical periods of shooting and heading. The significance of these facts becomes more apparent when it is pointed out that Milturum has approximately 8–10 days and Caesium 4–5 days longer stooling period than Marquis. While in this resistant stage, these varieties are able to survive periods of drought that ordinarily cause great damage to other sorts not possessing this delayed habit of growth. Talanov (43) has reported that the drought resistance shown by Milturum, when growing in certain drought areas of Russia, was due in part to a delayed stooling period.

Milturum and Caesium were also found to possess the capacity of developing their root systems rapidly in the early stages of development. This characteristic is of obvious importance when limited moisture is available at the time of seeding, since it allows the plant to establish itself more quickly in the soil. Furthermore, an early and well developed root system will enable a plant to weather early periods of drought more successfully. The importance of this in connection with soil drought is illustrated in Fig. 3. Reward, which is characterized by a scanty development of root system, was unable to survive a prolonged period of soil drought, while Caesium made complete recovery. Talanov (43) has reported that the root systems of Milturum and Caesium in their ultimate aspect are also characterized by profuse branching and great thickness.

Finally, the Russian varieties were found to possess a superior capacity to endure drought without permanent injury. Tumanov (44) has pointed out that Caesium possesses considerable resistance to soil drought. This investigator found that the wheat varieties that he tested fell into two distinct groups with regard to survival, following a prolonged exposure to soil drought, *viz.*, those with a survival of more than 80% and those with a survival of less than 50%. Caesium fell into the former group.

The results of the present study show Milturum and Caesium to be particularly resistant to atmospheric drought when tested in the earlier stages of development. They not only showed, as a rule, less foliage injury than the susceptible types, but made more rapid recovery. Milturum was superior to Caesium in both these respects.

Hardening of the plants prior to exposure to atmospheric drought, by subjection to soil drought or to limited periods of atmospheric drought, caused greater tolerance in both resistant and susceptible types. Hardening was reflected in a reduced leaf and culm injury, and in more rapid recovery as determined by general appearance of vigor. It was a noticeable feature of the work on hardening that unhardened plants, which did not appear to be unduly injured after exposure, were found to be much more stunted at maturity than corresponding hardened plants. This is well illustrated in Fig. 1, where the striking difference in height of hardened and unhardened plants of the Milturum variety is clearly indicated.

Varietal differences in susceptibility tended to be minimized when the plants were hardened before exposure to drought (See Tables VI–VIII).

It is thought that greater differences would have occurred had the exposures been longer so as to overcome somewhat the tolerating effect of hardening.

Percentage leaf loss was found to be a fairly reliable criterion of susceptibility when the plants were in either the stooling or early shooting stage. While close agreement existed in most cases between per cent leaf loss and per cent of culms and plants killed, one or two exceptions which are worthy of note occurred. With reference to Table VII, it will be noted that the leaf loss of the three varieties, Federation, Baart and Red Bobs No. 222, varied from only 30–37%, while the culm loss for some varieties varied from 0–23%. These data indicate that the most reliable index of susceptibility to atmospheric drought is obtained when per cent leaf loss is estimated in conjunction with per cent dead culms and per cent dead plants.

Leaf and culm loss were found to be of little value as a measure of susceptibility when the plants were tested in the advanced stages of soft and hard dough. At this time the leaves were very prone to desiccation, the phenomenon being not unlike that occurring in naturally ripening grain. The maturation processes in the culms were also hastened, and as they had lost their succulency at these stages it was difficult to distinguish between injured and non-injured culms. The degree of shrivelling of the kernels appeared to be the only reliable criterion of injury at these later stages.

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VITAMIN A REQUIREMENTS OF GROWING CHICKS

I. PRELIMINARY STUDIES WITH "REFERENCE" COD LIVER OIL[1]

BY JACOB BIELY[2] AND WILLIAM CHALMERS[3]

Abstract

In experiments conducted to determine the requirements of chicks for vitamin A up to eight weeks of age, vitamin A was supplied, in addition to the basal ration, by oral administration of Reference Cod Liver Oil at the rate of 25, 50, 75 and 100 International units per day. It was found that chicks receiving 75 units of vitamin A per day grew at a normal rate and did not show any signs of vitamin A deficiency. The chicks that received the basal ration only, showed symptoms of avitaminosis A at three weeks of age, while the group receiving 25 international units per day at eight weeks clearly had not received a sufficient supply of the growth factor.

Although the role of vitamin A in promoting normal growth and development of fowls was established over a decade ago (5), comparatively little is known about the quantitative requirements of growing chicks for this dietary factor. This note reports preliminary results obtained in feeding chicks graded quantities of vitamin A, using the International unit as the basis of measurement. Reference Cod Liver Oil (vitamin A) obtained from the Committee of Revision of the Pharmacopoeia of the United States of America (2) was used for this purpose. This oil is standardized to contain 3000 international units of vitamin A in one gram of the oil. The vitamin D content of the oil is not specified. In order to ensure an ample supply of vitamin D the ration was supplemented with irradiated yeast.

Experimental Methods

Day-old sexed S.C. White Leghorn cockerels were divided into five groups of 12 chicks each, except the control, which consisted of 21 chicks. They were placed in a battery brooder and fed when 24 hours old. Each tier of the battery brooder was 24 × 60 in. and divided into two compartments of equal size. One of the compartments in each tier was heated by three carbon filament lamps.

The chicks were fed a basal ration similar to that used by Elvehjem and Neu (4) in vitamin A determinations, except that irradiated yeast (70 D) served as a source of vitamin D instead of ultra-violet light. This ration was thought to be free of vitamin A, but in the light of the recent work of Giddings and Swim

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(6) on the vitamin A content of casein and yeast, it would appear that it contained traces of the vitamin. The basal ration and water were continually available to the birds. Analyses of the tibia of several control chicks showed that bone calcification was normal, the ash content being over 45%.

Four groups of chicks were given supplements of vitamin A once a week at the respective rates of 25, 50, 75 and 100 International units per day. By dilution with the calculated proportions of Wesson oil, the Reference Cod Liver Oil was made up into standard solutions containing the seven-day quotas of 175, 350, 525, and 700 international units per cubic centimetre. At the beginning of each week these solutions were fed directly into the crop by pipette in 1 cc. amounts. That the feeding of vitamin A once a week gives results comparable to daily administration has been shown by the work of Coward and Key (3) on rats.

The control chicks were fed the basal ration only.

Results

The average weekly weights of the chicks in the various groups are shown in detail in Table I, and graphically illustrated in Fig. I.

TABLE I

AVERAGE WEEKLY WEIGHTS OF ♂ CHICKS

Lot No.	Number of International units of vitamin A fed per day	Average weights								
		1-day-old, gm.	1 week, gm.	2 weeks, gm.	3 weeks, gm.	4 weeks, gm.	5 weeks, gm.	6 weeks, gm.	7 weeks, gm.	8 weeks, gm.
1	100	36.2	59.3	98.5	139.6	204.8	282.2	359.0	446.4	543.4
2	75	36.0	58.2	91.3	133.6	197.0	268.2	348.2	428.2	552.4
3	50	37.3	62.7	100.9	143.2	194.4	257.0	329.3	427.5	507.7
4	25	38.1	62.7	101.0	140.6	191.2	253.0	316.7	381.7	421.2
5	Control	34.8	58.1	87.1	115.9	151.3	176.8	209.3	387.0*	All dead

*2 chicks only.

The chicks in the control group began to show definite symptoms of avitaminosis A at three weeks of age. The growth rates of the control chicks are in accord with those reported by Elvehjem and Neu (4). The chicks that received the basal ration only, in all cases showed loss in weight* shortly before death. Post-mortem examination invariably revealed the presence of urates in the kidneys and ureters. Lesions in other organs were not observed as frequently as in the kidneys or ureters. The first mortality occurred at three weeks of age and the last during the eighth week. All the chicks in this group showed marked symptoms of vitamin A deficiency at five weeks of age. The ability of some of them to live beyond that period was probably due to individual reserves and the trace of vitamin A in the ration, apparently sufficient to support subnormal life.

The chicks receiving graded quantities of vitamin A grew at a fairly uniform rate up to four weeks of age. After the end of the fourth week the chicks

*The weights of the chicks that died during the week are not included amongst the average weekly weights shown in Table I and Fig. I.

which received 25 units of vitamin A began to lag in growth, so that by the end of the eighth week they weighed significantly less than the chicks receiving 50 International units. Similarly, the chicks which received 50 units grew at a somewhat slower rate than the ones which received 75 units, and the

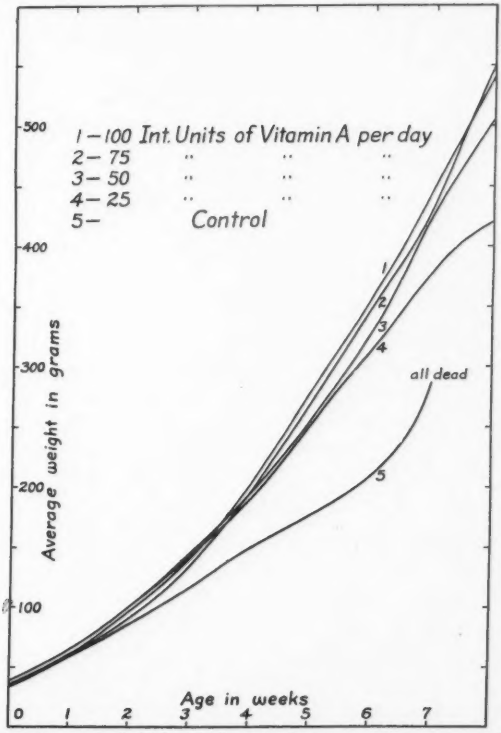

FIG. 1. *Growth curves of chicks which received various supplements of vitamin A.*

chicks which received 75 units grew at a slightly slower rate than the chicks which received 100 units. For some reason unaccounted for, those receiving 100 units of vitamin A did not show corresponding gains during the eighth week. A statistical analysis of the weights of the individual chicks at eight weeks of age is given in Table II. Table III shows the significance of the differences of the average weights of the groups at eight weeks of age.

TABLE II

STATISTICAL ANALYSIS OF THE WEIGHTS OF CHICKS AT EIGHT WEEKS OF AGE

Supplement	Mean weight	Standard deviation	Coeff. of var.
25 Int. units/day	421.2 ± 22.93	96.16 ± 16.21	22.83 ± 4.04
50 Int. units/day	507.7 ± 22.28	93.44 ± 15.76	18.41 ± 3.20
75 Int. units/day	552.4 ± 16.23	68.05 ± 11.48	12.32 ± 2.11
100 Int. units/day	543.4 ± 20.87	97.87 ± 14.76	18.01 ± 2.80

TABLE III

STATISTICAL SIGNIFICANCE OF DIFFERENCES BETWEEN WEIGHTS AT EIGHT WEEKS OF AGE

Supplements	Difference of means	Difference / Probable error	Significance of the difference
25 units: 50 units/day	86.5 ± 31.97	2.706	Probably significant
50 units: 75 units/day	44.7 ± 27.56	1.622	Not significant
75 units: 100 units/day	9.0 ± 26.44	0.3403	Not significant
25 units: 75 units/day	131.2 ± 28.09	4.671	Significant

With the exception of the chicks which received 25 units, none of the groups showed any symptoms of avitaminosis A at eight weeks of age. As a matter of fact, the chicks appeared to be well developed, well feathered out and were apparently normal. Those which received 75 and 100 units per day compared favorably in weight with the average weight reported by Buckner *et al.* (1) for chicks brooded in confinement. It is of interest to note that the average weights of the chicks receiving 75 to 100 units of vitamin A equalled those receiving an otherwise identical ration, used in vitamin D assays, in which yellow corn was substituted for the white corn (59%). A repetition of the experiment at a later date gave practically identical results.

Conclusions

In an experiment of this nature where the amount of vitamin A supplied to each individual is constant, a period of eight weeks is apparently long enough to differentiate the growth responses of the chicks fed 25 units from those fed the larger unitages.

Had the oil been added to the mash in different percentages, the difference in the growth of the several lots of chicks would not have become so distinctly evident, since the greater the consumption of feed, the greater correspondingly the amount of vitamin A consumed. It may be concluded that the administration of 75 international units of vitamin A per day would ensure normal growth and protect chicks up to eight weeks against any symptoms of vitamin A deficiency. With the rate of growth obtained in this experiment, 100 units of vitamin A per day would appear to be above the immediate normal requirements of chicks up to two months of age.

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